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Award date:
1987

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CARDIOVASCULAR CONTROL BY CENTRAL BETA-ADRENOCEPTORS IN THE RAT

Submitted by Jane McHowat
for the degree of PhD
of the University of Bath

1987

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ACKNOWLEDGEMENTS

I would like to thank my two supervisors, Dr. Peter Redfern and Mr. Tony Draper for the help and advice they have given concerning this thesis.

I am very grateful for all the assistance offered to me by the staff of the animal house at the University of Bath, and thank Dr. Paul Flecknell and Mr. Robin Buckingham for the advice given concerning the surgical techniques I employed during this study.

I thank the Science and Engineering Research Council for financial support for this study and also ICI plc for the many gifts of drugs.

Finally, I would like to thank my father for the aid and patience shown during the typing of this thesis, and both my parents for the continuing love and support I have received throughout my academic years.

SUMMARY.

1. Icv injection of isoprenaline in anaesthetised New Zealand normotensive rats caused a hypotension and tachycardia which appeared to involve both an interaction with central beta$_2$- adrenoceptors and a possible non-neuronal mechanism which may involve the release of a humoral transmitter into the bloodstream. These responses were attenuated to a greater extent by chronic oral dosage, rather than acute pretreatment, with propranolol.

2. Hypotension and tachycardia induced by icv injection of isoprenaline or clenbuterol in the conscious New Zealand normotensive rat were attenuated by central propranolol.

3. Injection of adrenoceptor agonists into the hypothalamus of the anaesthetised New Zealand normotensive rat indicated that activation of central alpha-adrenoceptors caused hypertension whereas hypotension was a result of an interaction with central beta-adrenoceptors. An injection into the posterior hypothalamus was more likely to produce hypotension than one into the anterior hypothalamus.

4. Icv injection of isoprenaline in anaesthetised Japanese Okamoto spontaneously hypertensive and Wistar Kyoto normotensive rats caused hypotension and tachycardia. Hypotension was greater and tachycardia was less in Japanese Okamoto rats. In both strains, pretreatment with propranolol attenuated or abolished these responses.

5. Hypotension and tachycardia induced by isoprenaline injected into the hypothalamus of anaesthetised Wistar and Japanese Okamoto rats was attenuated or abolished by pretreatment with propranolol.

6. Icv injection of radiolabelled isoprenaline or propranolol indicated that leakage of drugs to the periphery was much greater in conscious than anaesthetised New Zealand normotensive rats.

7. Responses observed following central administration of drugs depended upon a number of factors, including strain, site of injection, choice of anaesthetic and presence of anaesthesia.

8. This study concludes that central beta- adrenoceptors play a role in the maintenance of blood pressure and blockade of these adrenoceptors may prove to be pro-hypertensive.

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Chapter 1.

INTRODUCTION

INTRODUCTION

The aim of this study was to investigate the role of central beta- adrenoceptors in the maintenance of blood pressure and whether action at these receptors by beta-adrenoceptor blocking drugs could contribute to their blood pressure lowering effect when used for the management of hypertension.

Although beta- adrenoceptor blocking drugs are used in the management of a variety of disease states, the precise mechanism of their action remains unclear. Beta-adrenoceptor blocking drugs employed in the control of hypertension appear to exert multiple actions, including the possibility of an action within the central nervous system. In order to investigate this hypothesis of a central action of beta- adrenoceptor blocking drugs, injections were made directly into the central nervous system of hypertensive and normotensive rats and any change in blood pressure and heart rate were recorded.

In this introduction, a brief account of nervous control of blood pressure and the disease hypertension will be given to illustrate the sites of action of beta- adrenoceptor blocking drugs. A review of the literature regarding central administration of adrenoceptor agonists and

antagonists into several species will illustrate the discrepancy in results obtained by workers in this field of research. A summary of the role of the hypothalamus in cardiovascular regulation will be given as injections into the hypothalamus were made in this study. Finally, the spontaneously hypertensive rat as a model of human essential hypertension will be discussed.

1.1. Nervous control of blood pressure.

The efferent nerves supplying the cardiovascular system belong to the autonomic nervous system. The vasomotor nerves to the blood vessels are principally from the sympathetic division of the autonomic nervous system. Blood vessels, in general, are not innervated by the parasympathetic nervous system.

Almost all sympathetic vasomotor nerves are adrenergic, the transmitter noradrenaline producing vasoconstriction by acting on the alpha- adrenoceptors of the vascular smooth muscle. Many blood vessels, particularly those in skeletal muscle, are endowed with beta- adrenoceptors subserving vasodilatation, but these receptors are of the beta2- subtype and are relatively unresponsive to noradrenaline; adrenaline from the adrenal medullae has a greater effect on them.

The level of the systemic arterial blood pressure is detected by pressure sensitive receptors, or baroreceptors, located at strategic points in the arterial side of the circulation. Deviations from a previously stable level of blood pressure affect these baroreceptors and elicit reflexes influencing the activity of the heart and blood vessels so as to compensate for the deviation such that the blood pressure tends to return to its initial level.

1.2. Central cardiovascular control centres.

Afferent nerves from the carotid sinus and aortic arch baroreceptors enter the medulla oblongata and form a descending tract on each side termed the tractus solitarius. The primary afferent axons form synaptic connections with cell bodies in the nucleus tractus solitarius, which surrounds the caudal end of each tractus solitarius; the medial portions of these nuclei are fused. The axons of the cell bodies of the tractus solitarius project into the medullary reticular formation.

Stimulation of the ventromedial reticular area produces depressor responses which are due to increased cardiac vagal activity and inhibition of activity in cardiac, vasomotor and adrenal sympathetic nerves. Stimulation of the lateral and rostral reticular areas produces pressor responses which are a result of increased activity in

cardiac, vasomotor and adrenal sympathetic nerves and inhibition of cardiac vagal activity.

Efferent axons from neurones in the medial parts of the rostral reticular formation of the medulla oblongata join the reticulo-spinal tracts of the spinal cord and make synaptic connections with the cell bodies of preganglionic sympathetic neurones in the lateral horns of the thoracolumbar segments of the spinal cord. Neurones in the nucleus ambiguus within the ventromedial depressor area give rise to axons innervating preganglionic cardiac vagal neurones in the dorsal nucleus of the vagus.

The nucleus tractus solitarius receives a descending pathway from the neocortex which modulates the input from baroreceptors before the connections with the reticular cardiovascular centres. Thus the sensitivity of the baroreceptor reflexes may be affected from the highest level of the central nervous system.

There are two main pathways for the ascending projections of baroreceptor input. One originates from the nucleus tractus solitarius and ventromedial reticular depressor area and runs to the hypothalamus with further projections to the amygdaloid nucleus and to the region of the septum pellucidum between the fornix and the corpus callosum. The other pathway passes through the centromedian nucleus to

the thalamus and thence diverges to the anterior thalamic nucleus with further projections to the septum pellucidum, or to the dorsal thalamic nucleus with further projections to the fronto-orbital cortex. The interconnections between the hypothalamus and the reticular system of the medulla oblongata are concerned with the integration of the control of the cardiovascular system and are involved in the cardiovascular accompaniments of emotional reactions.

A close association exists between cardiovascular control centres and the distribution of noradrenaline containing neurones. Noradrenergic neurones concerned with integrative cardiovascular control ascend from the medulla oblongata to the hypothalamus, hippocampus and limbic system and cerebral cortex. Descending axons of the reticulospinal tracts arise from noradrenaline containing and serotonin containing neurones in the medulla oblongata.

The nucleus tractus solitarius possesses a high density of adrenaline containing nerve terminals. Ascending and descending adrenergic pathways exist in the central nervous system which are thought to be involved in cardiovascular control. The existence of an adrenergic, vasodepressor system has been proposed and the hypotensive action of clonidine has been attributed to the direct action of the drug on adrenaline receptors (Bolme et al, 1974; Fuxe et al, 1975).

This is a very brief outline of the role of the central nervous system in the control of blood pressure. For further information regarding central cardiovascular pathways and central adrenoceptors involved in cardiovascular control, the reader is directed to review articles by Dampney (1981), Day and Roach (1974b) and Spyer (1982).

1.3. Hypertension and its management.

Hypertension in humans is a common abnormality which is associated with a high degree of early mortality and morbidity. It is identified with resetting of homoeostatic mechanisms for blood pressure regulation at a substantially higher level than is expected in the healthy population. As this pressure load is carried for years, hypertrophy of the heart, accelerated sclerosis of blood vessels, and target organ damage occurs, particularly in the brain, kidney and heart. Systemic hypertension arises from a variety of disturbances in circulation, with either increased cardiac output, peripheral resistance, or both. Compensatory mechanisms are retained when hypertension has become fixed, but rather than adjusting pressure within a normal range, it is maintained at higher than normal pressure ranges.

Hypertension may be divided into two types:

Primary, or essential, hypertension, in which the underlying cause is unknown.

Secondary hypertension, in which the elevation of blood pressure is a consequence of another disorder or can be assigned to a clearly identifiable cause, such as phaeochromocytoma or renal failure.

Hypertension is usually classed into three increasingly dangerous states: mild, moderate and severe. In mild hypertension, there is no clinically detectable impairment of either the heart or the kidneys; blood pressure is higher than normal, but otherwise nothing seems to be wrong. Increasingly high blood pressures bring increased complications. In moderate hypertension the kidneys are not working efficiently and the heart is having to work harder in the face of increased total peripheral resistance. Finally, in severe hypertension, the heart becomes enlarged, further deterioration of the kidneys has occurred and total peripheral resistance is further increased.

The actual level of blood pressure at which hypertension can be said to exist is arbitrary, since blood pressure can vary considerably between individuals eg. it is normal for

blood pressure to increase with an increase in age. For practical purposes, it is only necessary to decide on the blood pressure at which antihypertensive therapy is to be instituted. This decision is based on the likely benefit to be obtained by lowering the blood pressure, the possible adverse effects of antihypertensive drug therapy and the presence of complications.

The prime purpose of antihypertensive treatment is to bring the blood pressure down to more normal levels. The idea is to lower the total peripheral resistance which should lower the load on the heart and improve blood flow through the kidneys. There is a wide variety of drugs which may be employed in the management of hypertension, practically all work through more than one mode of action. Usually, the first-line therapy used in hypertension is a drug which will increase the volume of urine produced, this also eliminates sodium from the body and lowers blood pressure. Other drugs which act at different sites in the body may also be useful if this first-line attack is ineffective.

These include various classes of drugs:

Calcium antagonists reduce vascular contractility by inhibiting the influx of calcium ions into the cell. They lower arteriolar resistance which leads to a fall in blood pressure. Verapamil has negative ionotropic and

chronotropic activity which may limit the reflex tachycardia that normally occurs as blood pressure falls, but other calcium antagonists affect the heart to a lesser degree.

Central alpha- adrenoceptor agonists are capable of passing through the blood-brain-barrier and stimulating central alpha$_2$- adrenoceptors in the medulla, decreasing sympathetic outflow. In the periphery, stimulation of presynaptic alpha$_2$- adrenoceptors invokes the negative feedback mechanism controlling the release of noradrenaline. The net effect is that the sympathetic vasoconstrictor influence is reduced, total peripheral vascular resistance decreases and blood pressure falls.

Alpha$_1$- adrenoceptor blockers selectively block vascular post-synaptic alpha$_1$- adrenoceptors, preventing the contractile response of the arteriolar resistance vessels to noradrenaline. Arterial pressure is reduced and there is little or no reflex tachycardia. This beneficial effect is probably due to a lack of effect on presynaptic alpha$_2$-adrenoceptors which leaves the negative feedback system intact and prevents excessive sympathetic activity.

ACE inhibitors inhibit the conversion of the relatively inactive angiotensin I to the very potent vasoconstrictor angiotensin II. They lower systemic arteriolar resistance

and increase the compliance of large arteries. The secretion of aldosterone is also reduced, so the kidneys excrete more sodium and water. Mean diastolic and systolic pressure falls in all grades of hypertension. Blood flow in the cerebral and coronary vascular beds (where autoregulatory mechanisms predominate) is maintained and they are without spasmolytic activity. Consequently cardiovascular reflexes are retained and postural hypotension is rare.

Adrenergic neurone blockers prevent the release of noradrenaline from nerve terminals in response to sympathetic stimulation. Cardiac output and peripheral resistance are lowered, venous capacitance is increased and blood pressure falls.

Rauwolfia alkaloids deplete noradrenaline from its storage sites in the adrenergic nerves. Sympathetic activity is reduced, peripheral resistance is lowered and blood pressure falls.

Vasodilators cause direct relaxation of arteriolar vascular smooth muscle with a consequent lowering in peripheral resistance and a fall in blood pressure. However, heart rate, stroke volume and cardiac output are increased through reflex sympathetic stimulation.

Beta- adrenoceptor blocking drugs represent one of the most important contributions to antihypertensive therapy. Since this study is concerned with their mechanism(s) of action, they will be discussed in greater detail.

1.4. The use of beta- adrenoceptor blocking drugs in the management of hypertension.

Following results published by Powell and Slater (1958) showing that dichloroisoprenaline reduced the depressor and vasodilator actions of isoprenaline, pronethalol was synthesised at ICI PLC and its properties were described by Black and Stephenson in 1962. The next beta- adrenoceptor blocking drug to be developed was propranolol, which was found to be more active than, and did not display the carcinogenic activity associated with, pronethalol (Black et al, 1965). One of the first reports on the blood pressure lowering action of propranolol in man was that of Pritchard and Gillam (1964).

Since propranolol was first marketed in Britain, many other beta- adrenoceptor blocking drugs have been developed in an attempt to achieve more specificity, less side effects, etc., whilst propranolol has remained the standard agent to which all others are compared.

Although beta- adrenoceptor blocking drugs have been used clinically for over 20 years in the management of hypertension, their precise mechanism of action is still unknown. It is known that the antihypertensive effect of these drugs is related to blockade of beta- adrenoceptors, since the dextro- isomers which do not bind to beta- adrenoceptors do not lower blood pressure (Rahn et al, 1974). Thus, the antihypertensive action of beta- adrenoceptor blocking drugs depends on the attenuation of the effects of sympathetic stimulation through competitive antagonism of catecholamines at beta- adrenoceptors.

It is not appropriate here to give a detailed account of all the theories postulated for the mechanism(s) of the antihypertensive action of beta- adrenoceptor blocking drugs, but a short summary is useful.

Reduction in cardiac output.

A reduction in cardiac output by beta- adrenoceptor blocking drugs becomes more apparent when sympathetic tone is enhanced, such as during exercise (Johnsson et al, 1969). However, a reduction in cardiac output does not provide a sufficient explanation for the antihypertensive effect of beta- adrenoceptor blockade because this is immediately decreased whereas a reduction in arterial blood pressure is delayed. This, together with the fact that all

beta- adrenoceptor blocking drugs have similar antihypertensive effects but differing effects on cardiac output led Tarazi and Dustan (1972) to assume that their antihypertensive action could be the result of adaptive changes in vascular flow resistance resulting from persistently diminished cardiac output.

Resetting of baroreceptors.

Prichard and Gillam (1969) suggested that the continuously reduced stimuli following blockade of the cardiac beta-adrenoceptors would lead to a resetting of the baroreceptors at a lower level. This hypothesis was supported by animal studies (Booker et al, 1977; Tuttle and McCleary, 1978), but conflicting results in hypertensive patients that baroreceptor sensitivity remained unchanged by beta- adrenoceptor blockade suggest that the involvement of baroreceptors is uncertain.

Restoration of vascular relaxation sensitivity.

Although this hypothesis is based on many assumptions, it is consistent with clinical findings, particularly the slow onset and disappearance and the uniform pattern of the antihypertensive effect of all beta- adrenoceptor blocking drugs.

Amer (1977) proposed that chronic blockade of vascular beta- adrenoceptors would allow the adenylate cyclase relaxation complex to recover from diminished responsiveness to known mediators such as histamine and prostaglandins, and thus counteract unopposed vasoconstrictor tone. In addition to this, chronic beta-adrenoceptor blockade has been shown to increase the number of beta- adrenoceptors and may enhance the responsiveness to endogenous vasodilators.

Inhibition of renin release.

The sympathetic nervous system both stimulates and is stimulated by the renin-angiotensin system (Passo et al, 1971), and inhibition of renin release by a beta-adrenoceptor blocking drug should reduce the activity of both systems by breaking this vicious circle (Buhler et al, 1975). However, in several studies, no correlation between plasma renin values and the extent of blood pressure reduction has been demonstrated (Morgan et al, 1975; Pederson and Kornerup, 1977).

Presynaptic inhibition of sympathetic transmission.

Stimulation of presynaptic beta- adrenoceptors is thought to facilitate noradrenaline release. Thus blockade should inhibit the release of neurotransmitter and reduce cardiac,

renal and vasoconstrictor nerve transmission as a whole.

Presynaptic beta- adrenoceptors have been implicated in the pathogenesis of hypertension. Adrenaline, the implicated natural stimulant of presynaptic beta- adrenoceptors, has been shown to enhance the release of noradrenaline in the rabbit (Hedler et al, 1982) and it causes hypertension in the rat, an effect that can be prevented by beta-adrenoceptor blockade (Majewski et al, 1981).

Many studies now suggest that the prejunctional beta-adrenoceptor is of the beta2 subtype (Majewski and Rand, 1984) and since beta1 adrenoceptor selective blockers are equally effective as antihypertensives, this prejunctional action could only be one of the many theorised.

A central nervous system effect.

This study is principally concerned with the possibility that the antihypertensive effect of beta- adrenoceptor blocking drugs may be via an action within the central nervous system, and so this theory will be examined more fully.

1.5. The central nervous system theory.

Beta- adrenoceptors in the brain play a role in modulating

central sympathetic outflow (Alexander et al, 1975; Bylund and Snyder, 1976; Day and Roach, 1973; Philippu and Kittel, 1977). A reduction of central output of sympathetic tone due to an action of beta- adrenoceptor blocking drugs at central beta- adrenoceptors could therefore be one of the mechanisms by which they initiate a reduction in vascular resistance in order to lower arterial pressure (Day and Roach, 1973; Lewis and Hoeusler, 1975; Offerhaus and van Zwieten, 1974; Reid et al, 1974).

However, the physicochemical properties of the different beta- adrenoceptor blocking drugs as determinants of ability to cross the blood-brain-barrier and to penetrate into the cerebrospinal fluid compartment vary considerably, whereas onset and magnitude of blood pressure reduction is similar (Neil-Dwyer et al, 1981; Taylor et al, 1981). The majority of available information on the central actions of beta- adrenoceptor blocking drugs has been derived by use of unconventional routes of administration of the drugs by local microinjections in the brain and by superfusion techniques. In experiments in which the drugs were given via more conventional routes, as in intact man, controversy still surrounds the relative importance of central as opposed to peripheral receptor antagonism.

1.5.1. Passage of beta- adrenoceptor blocking drugs across the blood-brain barrier.

There is no appreciable barrier to aqueous diffusion between cerebrospinal fluid and the extracellular fluid of brain tissue since the membrane lining the cerebral ventricles is permeable to hydrophilic substances of high molecular weight. Thus, the main factor governing whether a drug appears in the brain following peripheral administration is the passage across the blood-brain barrier.

The blood-brain barrier is not absolute and really represents a quantitative rather than a qualitative difference from other tissues in capillary permeability. The main structural feature underlying the relatively low permeability of most brain capillaries is the close application of the astrocytes to the basement membrane of the capillaries. Thus, a substance that penetrates to the interstitial fluid surrounding the neurones from the capillary blood has to penetrate the membranes of the astrocytes as well as the capillary endothelium. The barrier restricts protein-bound molecules and ions from gaining access to the central nervous system. The rate of entry of beta- adrenergic blocking drugs depends most closely on the lipid solubility of the non-protein-bound, unionised fraction of the drug.

The logarithm of the partition coefficient of a beta-adrenoceptor blocking drug divided by the organic and water phase of a mixture of octanol and water provides a measure of its lipophilicity. The value of this logarithm ranges from 20.20 for propranolol (very lipophilic) to 0.02 for atenolol (very hydrophilic). The more lipophilic a given beta- adrenoceptor blocking agent is, the easier it will equilibriate between the plasma compartment and the brain tissue. This may explain why less incidence of central nervous system side effects are encountered using less lipophilic beta- adrenoceptor blocking agents such as atenolol rather than the more lipophilic propranolol (Arendt et al, 1984).

Bianchetti et al (1980) found that after intravenous injection in rats, propranolol was rapidly distributed to various brain areas, closely related to the level of vascularisation of the brain. The greatest amounts were found in the cortex, followed by hippocampus, amygdala, hypothalamus and medulla. Since the blood in the brain had not been cleared prior to assay there may be some contribution from blood levels in this study.

Garvey and Ram (1975a) dosed rats orally for 14 days with propranolol, pindolol or sotalol, all three producing persistent peripheral beta- adrenoceptor blockade.

Determination of tissue distribution after 14 days found propranolol concentrated in the hippocampus and pindolol concentrated in the septum, with no significant central concentrations of sotalol.

Myers et al (1975) measured brain and plasma concentrations of propranolol in rabbits following intracerebroventricular (icv) injection and intravenous (iv) infusion of doses giving similar falls in blood pressure (0.2 mg/Kg icv and 1 mg/Kg iv). The concentrations of propranolol in the hypothalamus were of the same order (4 micrograms (mcg)/g icv and 3 mcg/g iv) at two hours, the time of the maximum hypotensive effect. The hypothalamus-to-plasma concentration ratio after iv infusion was 15:1. Brain-plasma ratio in man was measured and the average found to be 15:1, similar to that found in rabbits. The absolute values for hypothalamic propranolol concentration were 1-9 mcg/g, also similar to those associated in the latter species.

Cruickshank et al (1980) noted the presence of propranolol, metoprolol and atenolol in cerebrospinal fluid following oral administration for 3-22 days. For both propranolol and metoprolol, high concentrations were found in the brain; the brain-plasma ratio was approximately 15:1. Atenolol appeared at much lower concentrations: the brain-plasma ratio was approximately 0.1:1.

Thus, there is good evidence that beta- adrenoceptor blocking drugs are able to gain access to the brain and that the proportion gaining access is dependant on lipid solubility.

1.5.2. Evidence for a central hypotensive action of beta-adrenoceptor blocking drugs.

In the search for the central sites of action of beta-adrenoceptor agonists and antagonists, conflicting results have been obtained, not least from the wide differences in species, injection sites and drugs examined. Some of the studies will now be discussed further to give the reader an outline of results obtained by other workers prior to this report. These have been collected into various sections, each dealing with a separate species.

Dog.

Bhargava et al (1972) reported that, in anaesthetised dogs, bradycardia with hypotension occurred following icv injection of noradrenaline (50-200 mcg), but tachycardia with hypotension occurred on icv injection of isoprenaline (100-200 mcg). The responses to icv injection of noradrenaline were blocked by prior icv injection of 10 mg phenoxybenzamine, whereas those to icv isoprenaline were

blocked by prior icv propranolol (2 mg). These responses were thought to be central effects since following bilateral vagotomy, removal of both stellate ganglia and transection of the upper cervical cord the responses were abolished. They concluded from these results, that central alpha- adrenoceptors were concerned with bradycardia and central beta- adrenoceptors with tachycardia, but that both types were concerned with hypotension.

In unanaesthetised dogs, Conway and Lang (1974) showed that icv isoprenaline (50-200 mcg) produced tachycardia and a dose dependant hypotension. Icv injection of propranolol (600 mcg-2 mg) did not significantly alter heart rate or arterial pressure but abolished the responses to subsequent icv isoprenaline. However, the icv injection of propranolol also abolished tachycardia and hypotension produced by iv isoprenaline (1-2 mcg/Kg), suggesting that leakage of propranolol from the cerebral ventricle had occurred in the animals used in this study.

In anaesthetised dogs, Privitera et al (1979) demonstrated dose dependant decreases in plasma renin activity and blood pressure after intracisternally injected dl- propranolol, an effect which was not seen following intravenous injection of identical doses. Acute renal denervation abolished the renin suppressing action of intracisternal propranolol, this would not be expected if it were due to a

direct action of propranolol on the kidney following leakage from the cerebrospinal fluid. However, both d- and l- propranolol injected intracisternally significantly lowered plasma renin activity and blood pressure to a similar degree, indicating the effects may be a result of local anaesthetic activity rather than beta- adrenoceptor blockade. Both isomers have equivalent local anaesthetic potencies whereas the d- isomer has about 1/100th the beta-adrenoceptor blocking potency of the l- isomer (Barrett and Cullum, 1968).

Perfusion of propranolol throughout the entire brain ventricular system (25 mcg/Kg/min for 30 minutes) in anaesthetised dogs was found to decrease arterial pressure and heart rate and increase cerebrospinal fluid noradrenaline (Tackett et al, 1985). The hypotensive effect produced by propranolol was prevented by prior central administration of phentolamine (Tackett et al, 1981). The authors suggested that the hypotensive response to centrally administered propranolol results from an action of the drug to release noradrenaline which then stimulates central alpha- adrenoceptors to decrease peripheral sympathetic nerve activity and lower arterial pressure. This suggests that centrally administered propranolol lowers arterial pressure through a presynaptic action of the drug at noradrenergic nerve terminals in the medullary area of the brain. This was based on

observations that cerebrospinal fluid noradrenaline was elevated in all areas in which propranolol was administered; however, restriction of propranolol to the fourth ventricle resulted in a hypotensive response which closely paralleled that seen with whole brain perfusion of the drug.

Cat.

Icv injection of noradrenaline (40 and 80 mcg) consistently lead to sinus bradycardia and a slight depressor response in anaesthetised cats (Share and Melville, 1963). These doses induced marked pressor responses and cardioacceleration in centrally reserpinised, vagotomised cats. Icv injection of adrenaline (40 mcg) in centrally reserpinised cats induced significantly less pressor responses and cardioacceleration than did an equal amount of noradrenaline, but after spinal C2 section, the cardiovascular responses were similar to those observed with noradrenaline after spinal section.

Icv injections of picrotoxin were shown to activate central sympathetic mechanisms through a release of brain stem noradrenaline (Share and Melville, 1964), resulting in a marked pressor response and tachycardia in anaesthetised cats. Central pretreatment with dichloroisoprenaline did not significantly alter the pressor response (thought to be

alpha- adrenoceptor activation), but the degree of tachycardia was significantly reduced (thought to fall under the influence of beta- adrenoceptors). No blockade of tachycardia was observed following intravenous administration of dichloroisoprenaline (Share and Melville, 1965).

In 1966, Gagnon and Melville injected isoprenaline into the cerebral ventricles of anaesthetised cats and reported dose dependant decreases in blood pressure with tachycardia. Pretreatment with pronethalol significantly reduced these effects in both normal and vagotomised cats. These were totally abolished by spinal C2 section. They further demonstrated that the responses to isoprenaline were significantly reduced by icv propranolol (30 mcg) but not affected by icv phenoxybenzamine (2 mg). They suggested the possible existence of specific central nervous system beta- adrenergic-like mechanisms mediating similar types of peripheral autonomic functions (Gagnon and Melville, 1967).

Philippu et al (1971) labelled the cat hypothalamus with ^{14}C noradrenaline and found that superfusion with noradrenaline and adrenaline enhanced the release of radioactive amines from the hypothalamus and caused a dose dependant increase in peripheral blood pressure. Superfusion with phentolamine before superfusion with noradrenaline did not diminish the effect of noradrenaline

on blood pressure, except at high doses which tended to cause ventricular haemorrhage. The results favoured the assumption that noradrenergic neurones of the hypothalamus may be of importance for the central regulation of blood pressure.

Share (1973) investigated the effects of icv propranolol and sotalol on directly (electrical stimulation of the dorsal medullary reticular formation) and reflexly (bilateral electrical stimulation of the cut central ends of the ulnar nerves) induced pressor and tachycardic responses in anaesthetised cats. The beta- adrenoceptor blocking drugs significantly inhibited the rise in heart rate following both types of stimulation, but had no effect on the pressor responses. Sotalol has no significant membrane stabilising activity and thus central beta-adrenoceptor blockade was likely to be responsible for the observed effects. Possibility of leakage to the periphery by the injected drugs was eliminated since the tachycardic response to intravenous adrenaline was unaffected.

Sustained falls in blood pressure and heart rate following icv administration of several beta- adrenoceptor blocking drugs were reported in conscious normotensive cats by Day and Roach (1974a). The drugs used were dl- propranolol, l-propranolol, dl- alprenolol, pindolol, practolol, ICI 66082, sotalol and oxprenolol. A slight initial increase

in blood pressure and heart rate was attributed to a membrane stabilising effect since it could be mimicked by the local anaesthetics procaine and lignocaine and by d-propranolol and d- alprenolol, which are both devoid of beta- adrenoceptor blocking activity. In these injections, no fall in blood pressure or heart rate was observed. That the depressor and bradycardic responses were not mediated by an action of the beta- adrenoceptor blocking agents in the periphery following leakage from the cerebrospinal fluid was suggested by the lack of alteration of the responses to intravenous isoprenaline in these animals.

Day and Roach (1974b) further demonstrated in conscious cats dose related increases in blood pressure and tachycardia following icv isoprenaline or salbutamol. In some animals, hypotension and tachycardia was observed after icv isoprenaline. These effects were abolished after icv beta- adrenoceptor blocking drugs but were unaffected by alpha- adrenoceptor blocking agents. Dose related falls in blood pressure and heart rate produced by icv noradrenaline were abolished after icv phentolamine but unaffected by icv propranolol. Icv adrenaline produced complex responses in untreated animals, but typical alpha-adrenoceptor mediated effects were obtained after prior icv treatment with propranolol and typical beta- adrenoceptor mediated effects after icv pretreatment with phentolamine. They speculated that at least some of the useful clinical

properties of beta- adrenoceptor blocking drugs were a result of an action at central beta- adrenoceptors.

Philippu and Kittel (1977) and Philippu and Stroehl (1978) inserted a push-pull cannula into the posterior hypothalamus of anaesthetised cats, this was superfused through the cannula and electrically stimulated through its tip. Electrical stimulation elicited a frequency dependant pressor response and tachycardia. The responses were inhibited by superfusion with dose-dependant atenolol, practolol and metoprolol ($beta_1$- selective), propranolol and sotalol (non selective) and butoxamine ($beta_2$- selective). Superfusion with isoprenaline or tazolol ($beta_1$- adrenoceptor stimulant) led to a concentration-dependant enhancement in the pressor response, but salbutamol ($beta_2$- adrenoceptor stimulant) was ineffective. Thus, both $beta_1$- and $beta_2$- adrenoceptors were implicated in these studies and suggested a possible hypothalamic target for the hypotensive action of beta- adrenoceptor blocking agents.

Rabbit.

In pentobarbitone anaesthetised rabbits, adrenaline (10-200 mcg) and noradrenaline (50 mcg) produced a fall in systemic blood pressure and heart rate when injected into the lateral cerebral ventricle (Toda et al, 1969). The

responses induced by adrenaline were not significantly affected by bilateral vagotomy, but were abolished by transection of the spinal cord. Isoprenaline (50-200 mcg) caused a fall in blood pressure and an acceleration of heart rate, whereas 200 mcg phenylephrine caused a slight rise in blood pressure in association with a decrease in heart rate. In unanaesthetised rabbits, adrenaline produced pressor and cardiostimulatory effects followed by depressor and cardioinhibitory effects. This would suggest a centrally mediated hypotensive action of adrenaline in anaesthetised rabbits but a hypertensive action in unanaesthetised rabbits. However, anaesthesia induced by pentobarbitone is thought to preferentially block pressor centres in the brain. Gutman et al (1962) showed that stimulation in the hypothalamus, the mesencephalon and the medulla of rabbits resulted in a rapid rise in blood pressure. Following administration of pentobarbitone, the blood pressure changes were either decreased or changed to a hypotensive action. Thus, the difference in responses to adrenaline may be an effect of the presence of anaesthesia.

Reid et al (1974) reported an initial rise in blood pressure followed by a prolonged fall following icv injection of propranolol in conscious rabbits. The initial pressor response was thought to be a membrane stabilising effect, since it was mimicked by d- propranolol and procaine, this pressor effect was abolished by

pentobarbitone anaesthesia. Isoprenaline caused a transient fall in mean arterial pressure which was abolished by pretreatment with central propranolol (Dollery et al, 1973).

Anderson et al (1977) also observed an increase in mean arterial pressure, followed by a small fall, caused by 500 mcg propranolol icv in conscious rabbits. However, in contrast to Reid et al (1974), the same dose injected iv resulted in a lowering of mean arterial pressure greater than that following icv injection. This group demonstrated rapid leakage of propranolol from the cerebrospinal fluid to the bloodstream. Ten minutes after icv injection, there was significant blockade of cardiac beta- adrenoceptors for at least two hours, as determined from the degree of attenuation of isoprenaline induced tachycardia. This study consequently highlighted the problem of differentiating between central nervous and systemic mechanisms following icv injection.

In conscious rabbits, Korner et al (1980) showed that high plasma concentrations of propranolol lowered the threshold pressure for inhibiting renal sympathetic nerve activity. Similar plasma concentrations of propranolol had little effect on aortic nerve baroreceptor activity. The authors concluded that propranolol was acting centrally to 'reset' the renal sympathetic baroreflex.

Rat.

Lavy and Stern (1970) applied propranolol in powdered form (1000 mcg) into various central nervous system structures of the anaesthetised rat. They observed a fall in heart rate, especially from the anterior hypothalamus and reticular formation. Injection of isoprenaline (20 mcg) into the anterior hypothalamus gave rise to tachycardia. The authors suggested that an antagonistic effect of propranolol and catecholamines existed in the rat central nervous system.

Intracisternal injection of isoprenaline in anaesthetised rats caused a decrease in blood pressure, whereas noradrenaline produced a transient increase (Ito and Schanberg, 1974). Intracisternal propranolol produced a pressor response at low doses but this was converted to a depressor response as the dose was increased. The pressor response was antagonised by subsequent intracisternal injection of isoprenaline but potentiated by noradrenaline.

In 1974, Struyker Boudier et al showed that injection of noradrenaline (3-100 nmol) into the anterior hypothalamus of anaesthetised rats resulted in a dose related fall in blood pressure and heart rate. The depressor effects were mimicked by phenylephrine and clonidine and abolished by

phentolamine, suggesting that stimulation of alpha-adrenoceptors in the anterior hypothalamus caused a fall in blood pressure and heart rate. Noradrenaline injected into the ventricles also caused a fall in blood pressure and heart rate, but the magnitude was significantly reduced. Injections of noradrenaline into the posterior and medial hypothalamus induced no cardiovascular change, or only a small increase in blood pressure without affecting heart rate.

Adrenaline (0.3-30 nmol) was found to reduce blood pressure and heart rate when injected into the anterior hypothalamus of anaesthetised rats (Struyker Boudier and Bekers, 1975). Adrenaline was found to be 10 times more potent than noradrenaline in inducing these intrahypothalamic effects on cardiovascular parameters. Immediately after injection of adrenaline, a small increase in blood pressure was observed, this was not dose dependant and was thought to be caused by leakage of some adrenaline into the peripheral circulation.

The central antihypertensive effects of propranolol in the conscious spontaneously hypertensive rat were studied by Sweet and Wenger (1976). A transient increase in arterial pressure was produced by icv injection of both dl-propranolol and d- propranolol. This increase was followed by a significant lowering of blood pressure at 24 hours.

These responses were not mimicked by systemically injected propranolol nor icv injected procaine. It would appear from these results that the hypotension produced by icv propranolol was independent of peripheral beta-adrenoceptor blockade and membrane stabilising activity. However, the membrane stabilising activity could not be completely ruled out since propranolol is 3 times more potent as a local anaesthetic than procaine, and the authors did report a slight hypotension following icv procaine. If membrane stabilisation was involved, these results would correlate with the findings of Kelliher and Buckley (1970) in anaesthetised cats, but would differ from the results of Reid et al (1974) in rabbits and Day and Roach (1974a) in cats.

Ozawa and Uematsu (1975) studied the cardiovascular effects resulting from intracisternal injections of sympathomimetic amines in the anaesthetised rat. Noradrenaline showed variable blood pressure responses which were blocked by phentolamine. Isoprenaline caused a fall in blood pressure with tachycardia, which was reduced after treatment with propranolol. Adrenaline showed both centrally mediated alpha- and beta- sympathomimetic effects. Tyramine caused mixed blood pressure responses, presumably due to a release of noradrenaline and adrenaline, and these responses were partially blocked after treatment with phentolamine or propranolol. The authors suggested that both alpha- and

beta- sensitive adrenergic zones may exist on the vasomotor centre of the pons and medulla, and both noradrenaline and adrenaline might play a physiological role as the neurotransmitters controlling blood pressure in the rat.

In conscious spontaneously hypertensive rats, Nomura (1976) reported a dose dependant fall in blood pressure following icv isoprenaline which was neither blocked by propranolol nor practolol, but was blocked by phentolamine, suggesting that isoprenaline was exerting an effect at alpha-adrenoceptors. The beta2- adrenoceptor agonists, salbutamol and orciprenaline, caused a central pressor response which was blocked by propranolol. As these responses were significantly increased in spontaneously hypertensive rats, the author suggested the involvement of central alpha- and beta2- adrenoceptors in the hypertensive state.

Borkowski and Finch (1977) reported that, in conscious spontaneously hypertensive rats, adrenaline (1-20 mcg icv) caused a dose related fall in blood pressure and heart rate. Pretreatment with phentolamine (100 mcg icv) did not significantly antagonise the effects, while pretreatment with propranolol (100 mcg icv) completely abolished the bradycardia and reversed the hypotension. The responses to icv adrenaline were reproduced to a greater extent in anaesthetised animals (Borkowski and Finch, 1978), but they

were not antagonised by pretreatment with propranolol or oxprenolol. This points to a modification by anaesthesia of adrenaline induced responses and their antagonism by beta- adrenoceptor blocking agents. In a further study, they compared the cardiovascular effects of centrally administered clonidine and adrenaline in the anaesthetised spontaneously hypertensive rat. The data indicated that different central mechanisms were involved in mediating the hypotension and bradycardia induced by centrally administered clonidine and adrenaline, and did not support the theory that the hypotensive effects of clonidine were mediated by central adrenaline receptor activation.

Cohen et al (1979) reported a central hypotensive activity of d- and l- propranolol, pindolol and isoprenaline injected icv in anaesthetised rats. The response to d-propranolol was reduced, suggesting both a membrane stabilising and a beta- adrenoceptor blocking effect involved in the hypotension produced. In the case of beta-adrenoceptor blocking agents, the fall in blood pressure resulted from the simultaneous decrease of cardiac output and peripheral vascular resistance, whereas isoprenaline induced hypotension was due solely to a fall in peripheral

vascular resistance, the intensity of which was reduced by the increase in cardiac output.

The suggestion that a contribution of both beta-adrenoceptor blockade and membrane stabilising activity are involved in the effect of beta- adrenoceptor blocking drugs was supported by Allott et al (1982). The authors investigated the effects of beta- adrenoceptor blocking drugs (injected through a stainless steel cannula electrically insulated except for the tip) on the pressor response to electrical stimulation of an area immediately dorsal to the posterior hypothalamus in anaesthetised rats. They found that l-, dl- and d- propranolol inhibited the pressor responses but that the beta$_1$- adrenoceptor selective blocker, atenolol, did not. The l- isomer was more active than the racemate but only about 4 times more potent than the d- isomer, whereas d- propranolol has only about 1/100th the beta- adrenoceptor blocking potency of the l- isomer (Barrett and Cullum, 1968).

Peres-Polon and Correa (1984) observed a fall in blood pressure following icv injection of isoprenaline (1-20 mcg) in anaesthetised and conscious rats. Pretreatment with propranolol (40-200 mcg) partially antagonised the hypotension, whereas pretreatment with 40 mcg phenoxybenzamine or 100 mcg phentolamine potentiated the depressor response. They reported the possibility of a propranolol insensitive mechanism involved in the depressor effect of isoprenaline since, even at very high doses, propranolol was ineffective at reducing the maximal

depressor response to isoprenaline. The existence of a central alpha- adrenoceptor mediated pressor mechanism, opposing the beta- adrenoceptor mediated depressor mechanism, was highlighted because alpha- adrenoceptor blocking agents potentiated the hypotension produced by isoprenaline and central administration of such drugs caused a marked, long lasting reduction in basal blood pressure levels in anaesthetised rats.

Icv injection of noradrenaline (10-20 mcg) was found to reduce blood pressure in anaesthetised rats but to elicit hypertension in conscious rats (Correa et al, 1985). The pressor response in conscious rats was found to be blocked following hypophysectomy and following pretreatment with d(CH_2)$_5$Tyr(Me)AVP, a potent and specific vasopressin antagonist (Sawyer et al, 1981), suggesting an involvement of vasopressin in the cardiovascular response. Central pretreatment with histamine antagonists also blocked the pressor response to noradrenaline, favouring the idea of a histaminergic mechanism mediating the pressor response. From the results obtained, the authors suggested that two major systems were activated by icv administration of noradrenaline in the rat:

1. A pressor pathway, sensitive to anaesthesia, involving the release of a hypophyseal factor but not activation of the sympathetic nervous system. This pathway is mediated

by a histaminergic mechanism with the involvement of both H_1 and H_2 receptors.

2. A depressor pathway which is apparent under anaesthesia and masked in the awake animal.

Injections of adrenaline (10-20 mcg icv) were found to produce only depressor responses in both anaesthetised and conscious rats, whereas 20-100 mcg normetanephrine (an alpha- adrenoceptor agonist) produced only pressor responses in both groups of animals (Peres-Polon and Correa, 1987). They concluded that a direct correlation existed between alpha- and beta- adrenoceptor agonist potencies and the occurrence of pressor or depressor responses caused by icv administration of catecholamines. This was supported by the fact that propranolol blocked the depressor responses and phentolamine blocked the pressor responses induced by icv injection of catecholamines. It is possible that alpha- adrenoceptor mediated pressor responses predominate in awake animals, whilst a beta-adrenoceptor mediated depressor mechanism, less evident in the awake rat, is greatly facilitated by anaesthesia.

Summary.

Following a survey of the literature concerning injection

of adrenoceptor agonists and antagonists, it is apparent that there is a wide variability in the results obtained. This may be generally explained by the use of conscious or anaesthetised animals, choice of anaesthetic, route of administration and even location of an injection within a single ventricle.

In general, hypotension and bradycardia are observed following central injection of beta- adrenoceptor blocking drugs, although whether this is a result of blockade of central beta- adrenoceptors or a membrane stabilising effect is unclear. In addition, following injection into the cerebral ventricles, it is difficult to assess how much of the injected drug has leaked to the peripheral system and is blocking peripheral beta- adrenoceptors.

The effects of centrally applied adrenoceptor agonists presents no less a conflicting spectrum of responses. The presence of anaesthesia appears to mask pressor responses in some studies; Gutman et al (1962) showed that pressor responses were more susceptible to anaesthesia than depressor responses, and this was observed in some of the studies reported here.

For a tabulation of responses to centrally applied drugs in various species, the reader is directed to Philippu (1980).

1.6. Selective beta- adrenoceptor agonists and antagonists.

There are beta- adrenoceptor agonists and antagonists that are said to be selective for either $beta_1$- or $beta_2$- adrenoceptors. Although termed selective, these substances usually have a higher activity at one type of beta adrenoceptor with some residual activity at the other.

In this study, selective beta- adrenoceptor agonists and antagonists were used to differentiate the sub-type of beta- adrenoceptor involved in the responses observed. A short review of some of the properties of the newer molecules will be made since they are not as well known as other, well-documented drugs.

Xamoterol (ICI 118,587) was found to possess an approximate 100-fold selective affinity for $beta_1$- adrenoceptors (Mian et al, 1985). It increases heart rate by about 43% of the maximum increase produced by isoprenaline and is a competitive antagonist of the chronotropic and vasodilator effects of isoprenaline on the heart and blood vessels (Nuttall and Snow, 1982). Because of its high selectivity for beta1- adrenoceptors when compared to more standard $beta_1$- adrenoceptor agonists such as dobutamine, it was decided to use xamoterol in this study.

Clenbuterol was chosen as a selective beta2- adrenoceptor agonist because it is likely that it can pass into the brain following peripheral administration. It is highly lipophilic, and following chronic treatment with clenbuterol a significant decrease in the beta-adrenoceptor density of the cerebral cortex of the rat has been reported (Hall et al, 1980; Ordway et al, 1987). Clenbuterol has been shown to have a higher affinity for beta2- adrenoceptors (Waldeck and Widmark, 1985) and exerts local anaesthetic activity in high concentrations (Engelhardt, 1976).

ICI 118,551 is a selective beta2- adrenoceptor antagonist with an *in vitro* beta2/beta1- selectivity ratio of 123 and has a membrane stabilising action similar to that of propranolol (Bilski et al, 1983).

1.7. The role of the hypothalamus in cardiovascular regulation.

Cardiovascular responses were first elicited from the hypothalamus by Karplus and Kreidl (1909). Since then, the localisation of specific 'cardiovascular' sites in the hypothalamus and the course of descending hypothalamic pathways involved in cardiovascular regulation have been investigated in a large number of studies (for reviews, see Calaresu et al, 1975; Ciriello and Calaresu, 1977). However, the search for hypothalamic neurones specifically associated with cardiovascular responses has met with limited success, primarily because of the function of these neurones (Hayward, 1977).

High levels of propranolol have been detected in the hypothalamus following iv injection in conscious rabbits (Bakke et al, 1974), anaesthetised cats and dogs (Garvey and Ram, 1975b) and conscious rats (Elghozi et al, 1979). This suggests that the central hypotensive action of propranolol may be, at least in part, the result of an action in the hypothalamus.

The presence of beta- adrenoceptors in the hypothalamus was demonstrated by Philippu and Kittel (1977) and Philippu and Stroehl (1978) using anaesthetised cats. They concluded that both $beta_1$- and $beta_2$- adrenoceptors were present in

the posterior hypothalamus and that they were involved in the pressor response elicited by electrical stimulation of the hypothalamus.

Bogaert and Schepper (1979) showed that the hypotensive activity of the paraventricular nucleus was enhanced by clonidine, methyldopa and propranolol. Clonidine was thought to actively stimulate the neurones of the paraventricular depressor nucleus and elicit a direct central hypotension, whereas methyldopa and propranolol enhanced the depressor properties of the paraventricular nucleus.

Pardini et al (1986) isolated discrete areas of the rat hypothalamus which facilitated reflex bradycardia. Administration of atropine indicated that the facilitation was primarily parasympathetic in nature. This facilitation followed stimulation of the ventromedial or anterior hypothalamic areas.

Kanna and Yamashita (1985) demonstrated that stimulation of the paraventricular nucleus lead to facilitation and inhibition of neurones in the nucleus tractus solitarius. This confirmed earlier anatomical and electrophysiological studies on the reciprocal connection between the nucleus tractus solitarius and the paraventricular nucleus (Calaresu and Ciriello, 1980; Conrad and Pfaff, 1976;

Kannan and Yamashita, 1983). The results also gave support to the hypothesis that the paraventricular nucleus is involved in the neural control of the cardiovascular system.

Finally, the posterior hypothalamus has been implicated in the pathogenesis of hypertension in the spontaneously hypertensive rat. Winternitz et al (1984) showed that at 5 and 7 weeks of age, the noradrenaline content of the posterior hypothalamus was significantly greater in the hypertensive than in control Wistar-Kyoto rats. These changes occurred before there was a significant difference in blood pressure between the two groups. The increase in noradrenaline content occurred in the absence of a concomitant change in noradrenaline turnover, suggesting that in the spontaneously hypertensive rat the noradrenergic input to the posterior hypothalamus was increased. Electrical stimulation of the posterior hypothalamus of spontaneously hypertensive rats was shown to result in an exaggerated pressor response in comparison to both normotensive and DOCA-salt hypertensive rats (Juskevich et al, 1978; Takeda and Bunag, 1978). In addition, lesioning of the posterior hypothalamus of spontaneously hypertensive rats was found to result in a reduction in blood pressure that was significantly greater than the depressor effect of a comparable lesion in normotensive controls (Bunag and Eferakeya, 1976).

Taken together, studies in the spontaneously hypertensive rat suggest that abnormalities of central cardiovascular regulation involving the posterior hypothalamus play a role in the pathogenesis of hypertension in this particular strain.

1.8. The genetically hypertensive rat as a model of clinical essential hypertension.

Discoveries in the field of hypertension have often been the result of reasoning from the similarity of phenomena recorded in animals and man. However, most animal models of human disease are more or less bad approximations of a poorly understood derangement in man. Important differences have been shown to exist amongst the strains of genetically hypertensive rats in terms of their sensitivity to salt, the participation of blood pressure elevating and blood pressure lowering systems, and the time of onset of hypertension.

In 1963, Okamoto and Aoki introduced a new model of experimental hypertension that required no physiological, pharmacological or surgical intervention. This spontaneously hypertensive rat was developed by meticulous genetic (brother to sister) inbreeding that uniformly resulted in 100% of the progeny having naturally occurring

hypertensive disease (Okamoto and Aori, 1963; Okamoto et al, 1966). In the spontaneously hypertensive rat, elevated blood pressure was detected at birth when measured with the Survo-Null micropipette transducer system (Bruno et al, 1979). Hypertension in this strain was achieved within 3 generations. Since then, several expert panels have reported that the spontaneously hypertensive rat is an excellent model of experimental hypertension that could serve as a counterpart for clinical essential hypertension (Underfriend and Spector, 1972).

In the spontaneously hypertensive rat, hypertension is 'clinically' very similar to essential hypertension in man. Both have their apparent onsets very early in life. Their elevated arterial pressure is mediated through a slow and progressively increased total peripheral resistance which demands cardiac and vascular adaptation. Eventually, cardiac failure, strokes and renal lesions result in a shortened life span by about one-third in both forms of hypertension. In both genetic diseases (especially hypertension in the rat) neural mechanisms seem to predominate in the early stages, whereas in the later and more complicated phases, structural, renal, endocrine, humoral and metabolic mechanisms, including some less well studied mechanisms (such as kallikrein-kinin, prostaglandins and vasopressin) may also participate. In both forms of hypertension there seems to be susceptibility

for aggravation of the disease by excess dietary sodium, stress and other environmental factors. Also, both naturally occurring diseases are responsive to antihypertensive agents. (For a detailed review, see Trippodo and Frohlich, 1981).

McGiff and Quilley (1981) argued that the spontaneously hypertensive rat was not the closest animal model for essential hypertension in man. This argument was based on the potential importance of a blood pressure lowering mechanism observed in most species, including man, intimately related to renal prostaglandins. This mechanism in the rat rather than being antihypertensive, may contribute to elevation of blood pressure.

The fact remains that the causes of human essential hypertension and genetic hypertension in the rat are enigmas, and so extrapolation from studies in the spontaneously hypertensive rat to the human disease may be inexact.

The majority of studies on hypertensive rats have been carried on the Japanese Okamoto strain, but there are many hypertensive strains available. The New Zealand strain of Smirk seems to be most similar to the Japanese strain but has not been studied as broadly (Smirk and Hall, 1958; Phelan, 1968; Phelan and Simpson, 1987). This strain of

genetically hypertensive rat is derived from a non-inbred closed colony of albino rats. Matings were made between animals with above average blood pressures and it was found that the increase in blood pressure was about 2 mmHg per generation for the first 20 generations of inbreeding. In this respect, these contrast strongly with the Japanese Okamoto strain, in whom blood pressure increased more rapidly in the first three generations of inbreeding.

In summary, it would appear that the hypertensive rat model is a good model for the study of hypertension, as long as the following are remembered:

1. It is unlikely that both forms of naturally occurring hypertension are identical expressions of genetically determined hypertensive disease.

2. Both forms of hypertension are polygenic in origin and are influenced by environmental factors.

3. Since the control of normal arterial pressure in both man and rat is multifactorial, it follows that certain pressor mechanisms may well operate in one form of genetic hypertension that do not necessarily occur in the other.

4. Owing to the well grounded physiological concept that as one regulatory factor becomes altered other homoeostatic

mechanisms must become involved secondarily, it follows that similar adoptive alterations in regulatory mechanisms necessarily will occur in both forms of genetic hypertension.

1.9. Aims of the thesis.

This study was designed to examine further the possible role of beta- adrenoceptors in the central regulation of blood pressure and thus test the hypothesis of a central mechanism of action contributing to the antihypertensive action of beta-adrenoceptor blocking drugs.

In this investigation, the effects of centrally injected beta- adrenoceptor blocking drugs on the cardiovascular responses to central administration of alpha- and beta-adrenoceptor agonists were observed in the rat. The investigation was conducted using the following methods:

1. Injection of beta- adrenoceptor blocking drugs and beta- adrenoceptor agonists into the cerebral ventricles of conscious and anaesthetised rats.

2. Injection of alpha- and beta- adrenoceptor agonists into the hypothalamus of the anaesthetised rat with previous injection of beta- adrenoceptor blocking agents into the cerebral ventricle.

3. Long term oral dosing with a beta- adrenoceptor blocking agent, followed by central administration of a beta- adrenoceptor agonist.

4. The use of hypertensive and normotensive anaesthetised rats to examine any alteration in responses owing to the presence of hypertension.

In addition, the study attempted to quantify the amount of leakage of drug to the periphery following central administration, and hence try to differentiate between peripherally and centrally mediated effects following central injection.

Chapter 2.

MATERIALS AND METHODS.

2.1 SURGICAL TECHNIQUES

2.1.1 Injection of drugs into the left lateral cerebral ventricle of anaesthetised New Zealand normotensive rats.

Male New Zealand normotensive rats, weighing 180 - 200g, were anaesthetised with a mixture of Hypnorm/Hypnovel i.p. (Flecknell and Mitchell, 1984) such that each rat was injected with 5mg midazolam hydrochloride, 10mg fluanisone and 0.315mg fentanyl citrate per Kg body weight. When unconscious, the rats were placed on a heated blanket (Bioscience Ltd.), which maintained body temperature at 37°C by means of a rectal probe.

The trachea was cannulated to facilitate artificial respiration if required. The left carotid artery was cannulated with a polyethylene tube (ref: 200/300/030, Portex Ltd.) connected to a physiological pressure transducer (Gould), the whole being filled with heparinised saline (1000 units heparin/ml, 0.9% w/v sodium chloride). The transducer was coupled to a strain gauge coupler type 7179 (Narco Biosystems Inc.) and the output was fed to a 3-channel pen recorder (Physiograph Mark III, Narco Biosystems Inc.) and to a Biotachometer coupler type 7302 (Narco Biosystems Inc.) which derived heart rate from the blood pressure pulse. The right jugular vein was cannulated with a saline-filled polyethylene tube

(ref: 200/300/020, Portex Ltd.) for the intravenous injection of drugs.

The head of the rat was placed in a small animal stereotaxic instrument (DKI 900, David Kopf Instruments). The head was manoeuvred such that the ear bars lay in the external auditory meatus of the ear. The head was then centralised within the frame by reference to the calibrations on the ear bars. The upper teeth were hooked over the incisor bar and the nose clamp was gently tightened.

The atlas of Konig and Klippel (1963) was used for injections into the cerebral ventricle of anaesthetised rats. For this atlas, the incisor bar is adjusted to 2.4mm below the interaural line (an imaginary line passing through the centre of the ear bars).

Location of the cannula in the left lateral cerebral ventricle.

The stereotaxic instrument consists of a rigid metal frame on which is mounted a carrier which can be finely controlled in three planes (See figure 1). Prior to injection of drugs, the tip is located at stereotaxic zero, this being the mid-point of the ear bars. The co-ordinates at this point are recorded in the following planes:-

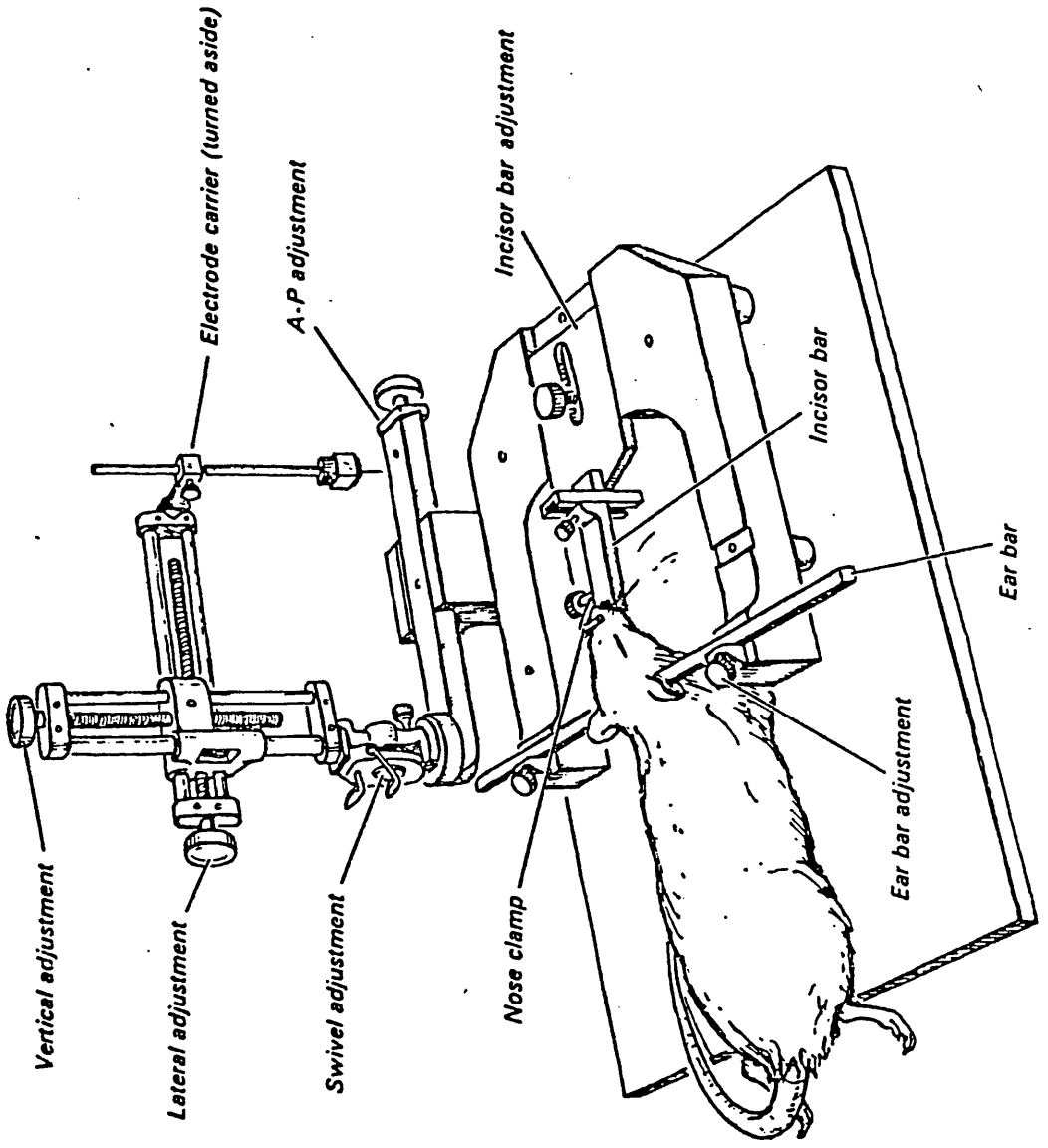

Figure 1. Position of the rat correctly mounted in the David Kopf small animal stereotaxic instrument. (Reproduced without permission from Pellegrino and Cushman, 1971)

AP (anterior-posterior)

L (lateral)

H (horizontal)

The rat brain atlas of Konig and Klippel has an arbitrary horizontal plane zero of 4.9mm above the inter-aural line; this must be added to the horizontal measurement taken from stereotaxic zero before the atlas co-ordinate may be calculated (see Pellegrino and Cushman, 1971 for a detailed discussion of the stereotaxic technique).

After the head of the animal had been mounted in the stereotaxic frame, the skull was exposed by a midline incision extending about 20mm back from the eyes. The underlying tissue adhering to the skull was scraped away.

The tip of the cannula was manoeuvred to the position on the skull directly over the area to be injected, and a hole was made in the skull using a model drill (Precision Petite). The following co-ordinates were used to locate the tip of the cannula in the left lateral cerebral ventricle according to the atlas of Konig and Klippel:-

AP +3.29mm

L -4.40mm

H -0.40mm (see Figure 2)

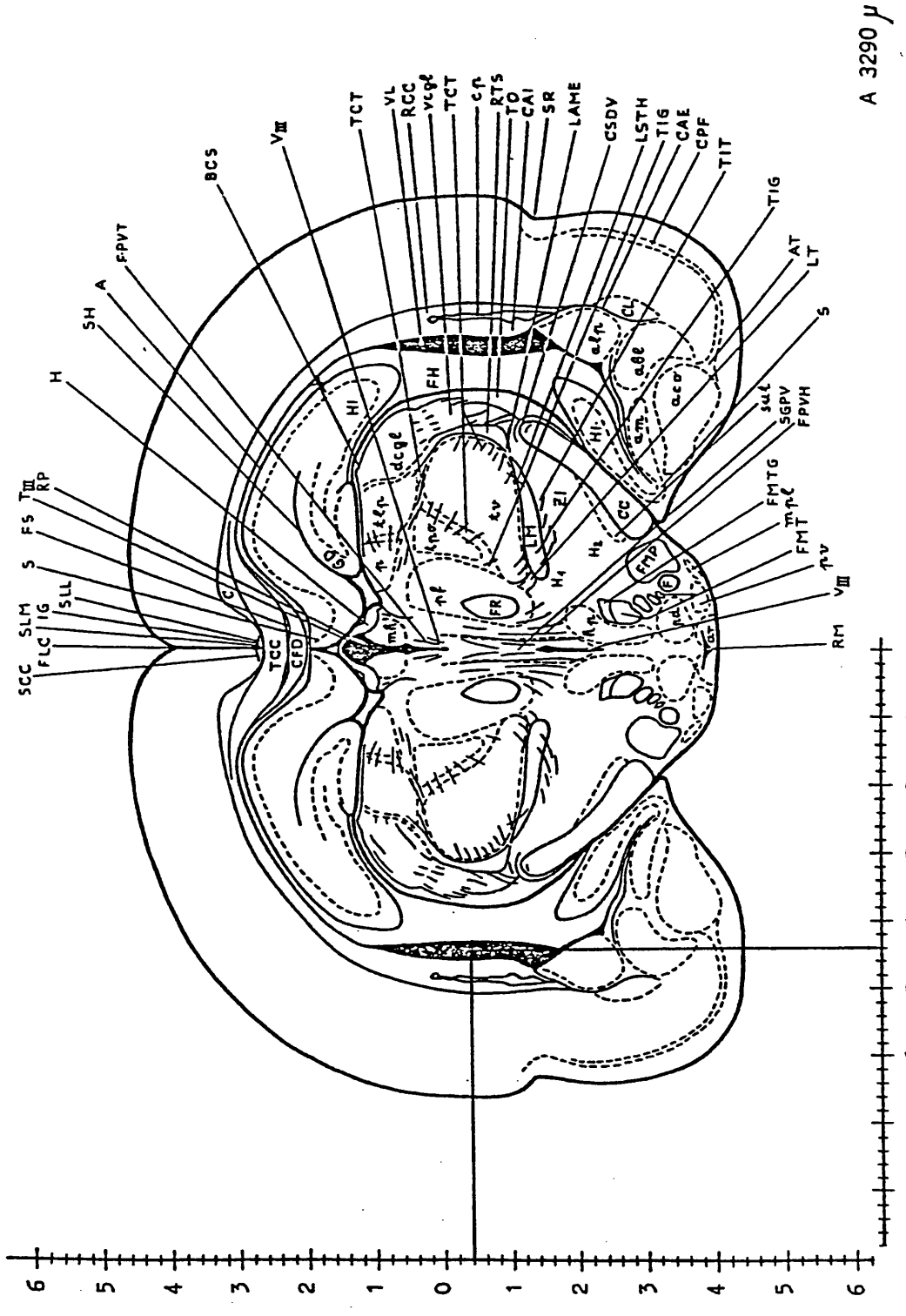

Figure 2. Site of injection into the left lateral cerebral ventricle in anaesthetised rats. (Reproduced without permission from Konig and Klippel, 1963)

The cannula was attached to a length of polythene tubing (ref: 800/100/140/100, Portex Ltd.) which had previously been filled with a solution of the drug to be injected. A fixed volume of solution was injected using a microlitre syringe according to the appropriate dosing schedule. Following injection, blood pressure and heart rate were monitored for 20 minutes.

2.1.2 Injection of drugs into the hypothalamus of the anaesthetised New Zealand normotensive rat.

Male New Zealand normotensive rats, weighing 180 - 200g, were surgically prepared as in Section 2.1.1. The following co-ordinates were used to locate the tip of the cannula in the anterior nucleus of the hypothalamus according to the atlas of Konig and Klippel:

AP +6.00mm
L -0.74mm
H -2.40mm (See figure 3)

The following co-ordinates were used to locate the tip of the cannula in the posterior nucleus of the hypothalamus according to the atlas of Konig and Klippel:

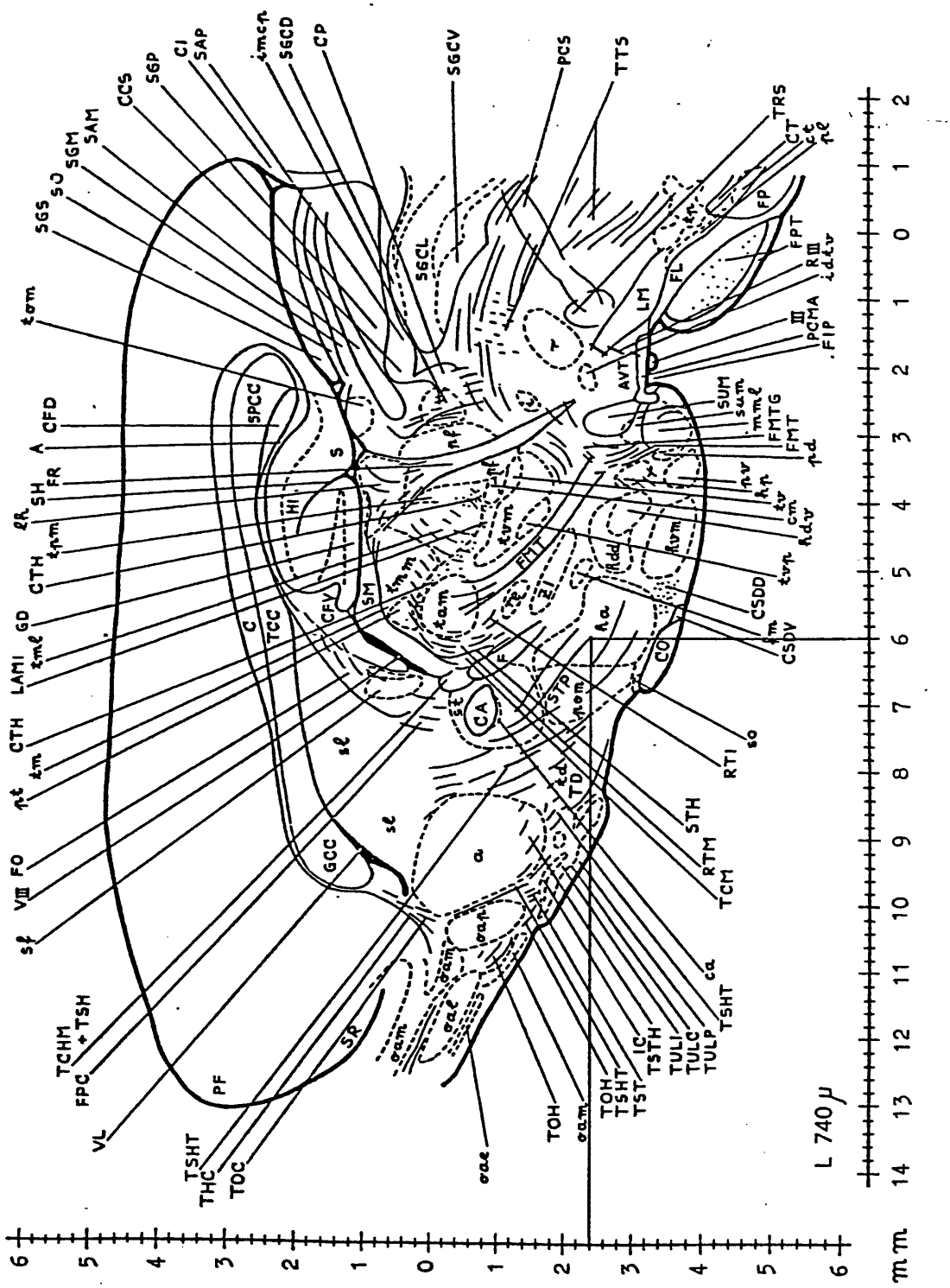

Figure 3. Site of injection into the anterior nucleus of the hypothalamus in the anaesthetised rat.
(Reproduced without permission from Konig and Klippel, 1963)

AP +3.50mm

L -0.40mm

H -2.40mm (See figure 4)

Where injections were made into both the cerebral ventricle and the hypothalamus, both holes were drilled through the skull prior to any injection of drugs.

2.1.3 Injection of drugs into the left lateral cerebral ventricle of anaesthetised Wistar rats.

Male Wistar rats (University of Bath strain), weighing 180 -220g, were anaesthetised with either Hypnorm/Hypnovel i.p. or sodium thiobutobarbitone 150mg/Kg i.p. (Inactin,BYK). The surgical procedure was as in section 2.1.1.

The tip of the cannula was located in the left lateral cerebral ventricle at: AP +3.29, L -4.40, H -0.04mm (Konig and Klippel, 1963).

2.1.4 Injection of drugs into the hypothalamus of anaesthetised Wistar rats.

Male Wistar rats (University of Bath strain), weighing 180 - 200g, were anaesthetised with sodium thiobutobarbitone 150mg/Kg (Inactin, BYK). These were then surgically

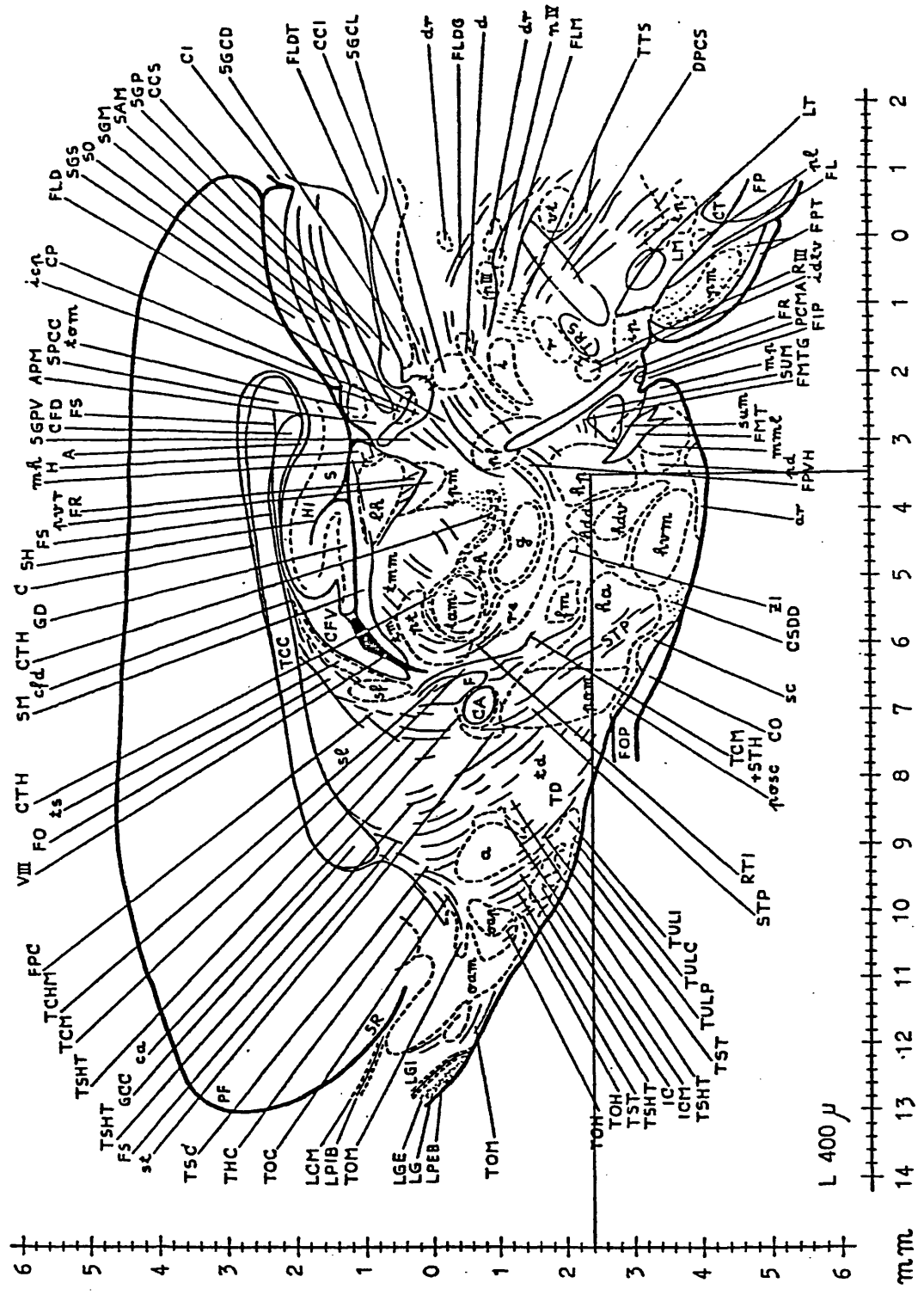

Figure 4. Site of injection into the posterior nucleus of the hypothalamus in the anaesthetised rat.
(Reproduced without permission from Konig and Klippel, 1963)

prepared as in section 2.1.1. The tip of the cannula was located in the anterior hypothalamus at AP +6.00, L -0.74, H -2.20mm (Konig and Klippel, 1963). The tip of the cannula was located in the posterior hypothalamus at: AP +3.50, L -0.40, H -2.20mm (Konig and Klippel, 1963).

2.1.5 Injection of drugs into the cerebral ventricle of anaesthetised Japanese Okamoto spontaneously hypertensive rats.

Male Japanese Okamoto rats, weighing 180 - 220g, were anaesthetised with either Hypnorm/Hypnovel i.p. or sodium thiobutobarbitone 100mg/Kg i.p. (Inactin, BYK). The surgical procedure was as in section 2.1.1. The tip of the cannula was located in the left lateral cerebral ventricle at: AP +3.29, L -4.40, H -0.04mm (Konig and Klippel, 1963).

2.1.6 Injection of drugs into the hypothalamus of anaesthetised Japanese Okamoto spontaneously hypertensive rats.

Male Japanese Okamoto rats, weighing 180 - 200g, were anaesthetised with sodium thiobutobarbitone 100mg/Kg i.p. (Inactin, BYK). These were surgically prepared as in section 2.1.1. The tip of the cannula was located in the anterior hypothalamus at: AP +6.00, L -0.74, H -2.20mm (Konig and Klippel, 1963). The tip of the cannula was

located in the posterior hypothalamus at: AP +3.50, L -0.40, H -2.40mm (Konig and Klippel, 1963).

2.1.7 Injection of drugs into the cerebral ventricle of conscious New Zealand normotensive rats.

2.1.7.1 Manufacture of cannulae.

The cannula for arterial blood pressure recording was made by joining together approximately 19cm of polythene tubing, internal diameter 0.4mm and external diameter 0.8mm (ref: 800/100/140/100, Portex Ltd.) to a 5cm length of polythene tubing, internal diameter 0.28mm and external diameter 0.61mm (ref: 800/100/100/100, Portex Ltd.), using the heat of a soldering iron. A single strand of copper wire, external diameter 0.2mm was passed through the tubes at the point of the join to maintain tubular patency during the heating procedure. The join was checked by filling the tubes with purified water before passing a small ring of polycarbonate film over the length of the larger gauge tubing down to the level of the join where the surface of the tubing had been widened by the heating process. The narrower bore tubing was the bent into a J-shape in near-boiling water close to the join.

The intravenous cannula was made from a single 20cm length of polythene tubing, internal diameter 0.4mm and external

diameter 0.8mm (ref: 800/100/140/100, Portex Ltd.). This was then bent into a J-shape approximately 5cm from one end, and a "stop" was manufactured close to the bend by attaching a small piece of autoclave tape to the tubing. (See figure 5 for a diagrammatical representation of the cannulae.)

2.1.7.2. Implantation of arterial and venous cannulae

Male New Zealand normotensive rats, weighing 180-230g, were anaesthetised with a mixture of Hypnorm/Hypnovel i.p., such that each received 4mg midazolam hydrochloride, 8mg fluanisone and 0.252mg fentanyl citrate per Kg body weight. When unconscious, the rat was shaved vertically over the abdomen and dorsally at the base of the neck. These areas were cleaned with chlorhexidine gluconate solution 0.05% (Hibitane, ICI P.L.C.). Midline incisions were made at the base of the neck (approximately 5mm long) and the abdomen (approximately 4cm long) and the abdominal muscles were cut along the line of the media alba. The intestines were exteriorised and covered with gauze soaked in saline. A stainless steel trochar (15cm long, internal diameter 1mm) was pushed gently and vertically through the psoas muscle [illegible] to exteriorise at the incision at the back of the neck. The cannulae were then passed through the trochar and the trochar removed. The intraarterial cannula was filled with heparinised saline

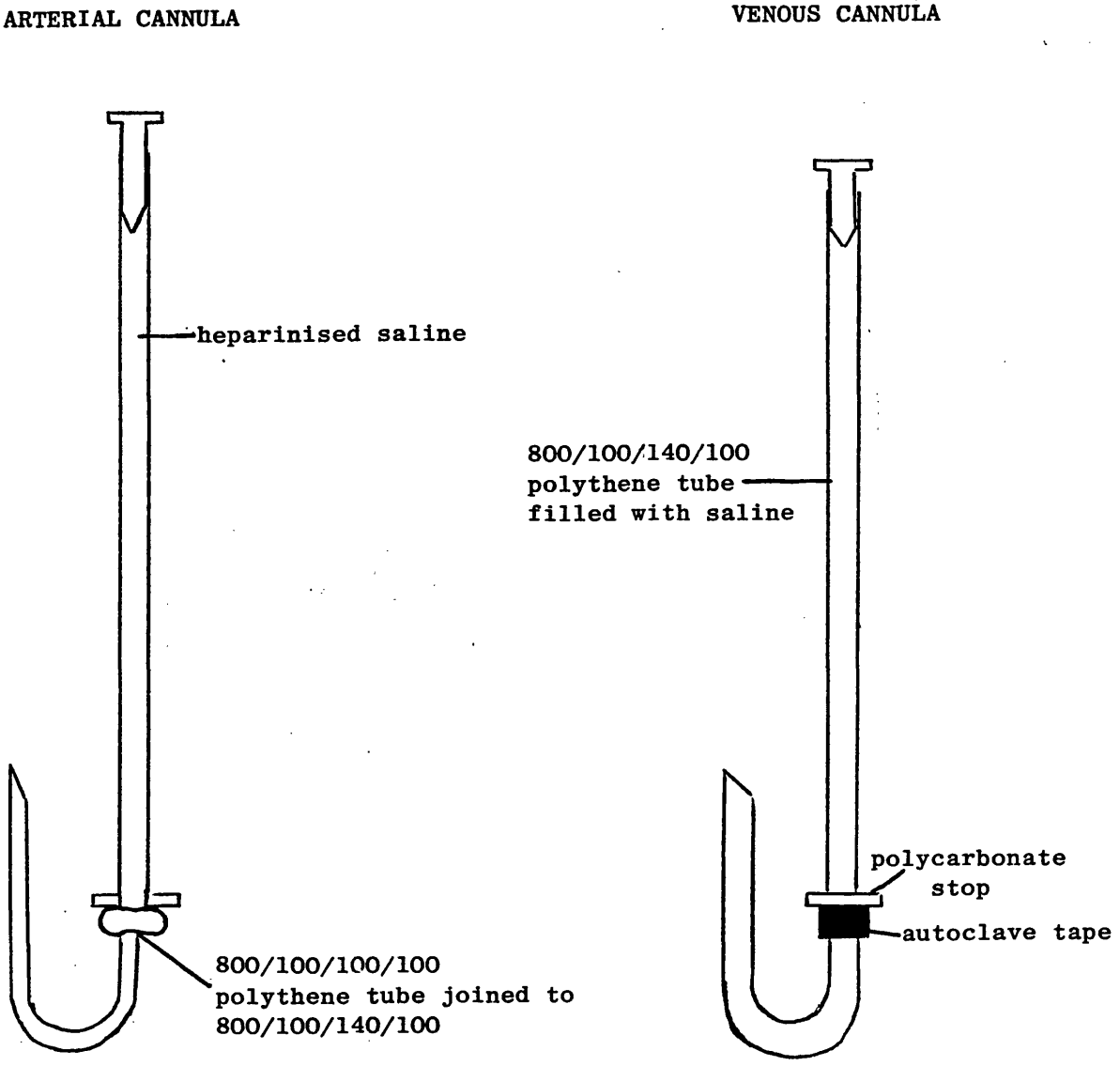

Figure 5. Construction of polythene cannulae for intraarterial and intravenous cannulation in the conscious rat.

(1000 units heparin/ml), the intravenous cannula being filled with non-heparinised saline. Each was anchored to the psoas muscle with a single stitch.

The connective tissue surrounding the abdominal aorta was gently separated with cotton wool and the abdominal aorta was clipped at a level rostral to the iliolumbar artery. A small hole was made in the aortic wall with a 25G hypodermic needle at a level directly opposite the suture holding the cannula in the psoas muscle. The tip of the cannula was inserted into the hole, the artery clip was removed and the cannula pushed gently further along the artery such that approximately 2cm of tubing was inside the vessel

The end of the intravenous cannula was cut to form a sharp tip and this was pushed gently through the connective tissue and vena cava wall into the vessel lumen at a level such that the venous cannula sat just below the arterial cannula (See figure 6).

The incisions in the abdominal muscle and overlying skin were closed with surgical silk sutures. The neck incision was closed around the exteriorised cannulae which were in turn trimmed to a length of approximately 2cm. The ends were sealed by inserting a 12mm stainless steel pin.

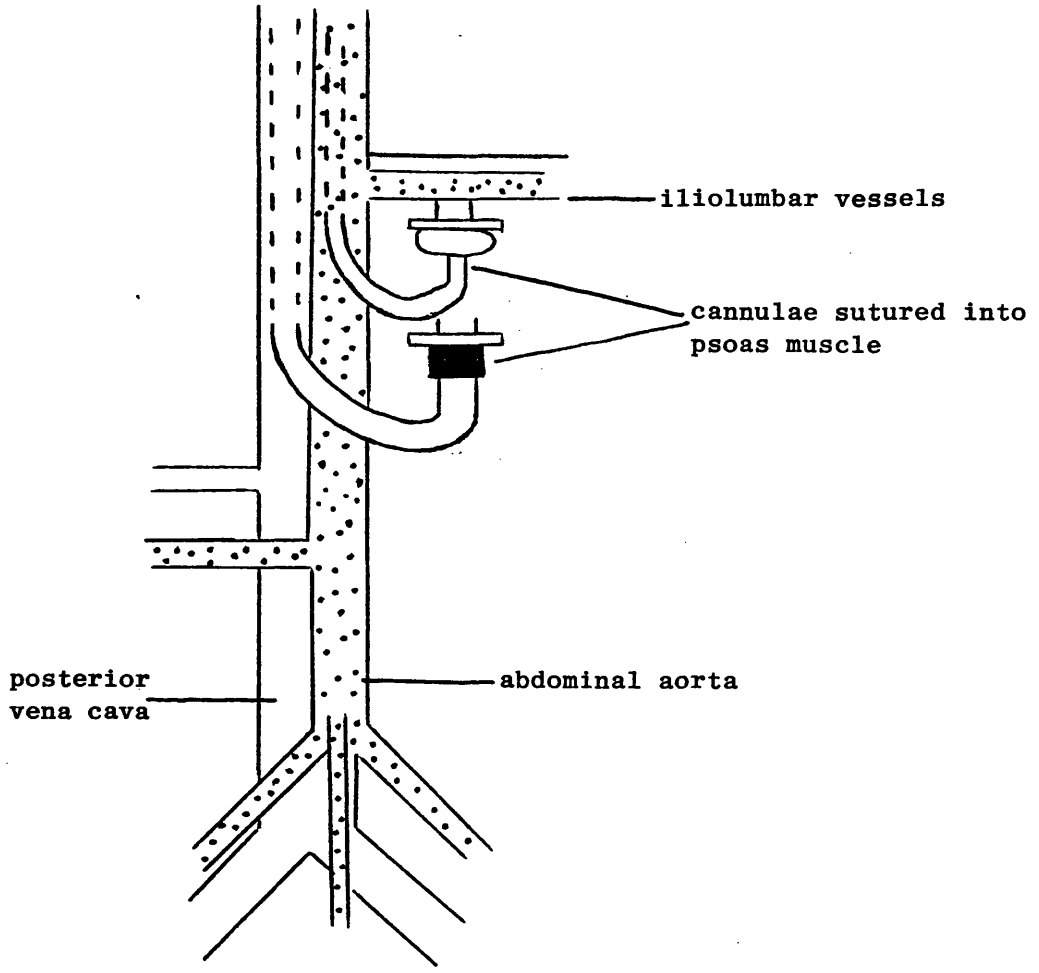

Figure 6. Cannulation of abdominal blood vessels.

The rats were then left to recover on a heated blanket for 24 hours. After the surgical procedure, the rats were housed individually to prevent chewing of the catheters by cage companions.

The cannulae were flushed daily, the arterial cannula with heparinised saline (200 units/ml heparin), the venous cannula with non-heparinised saline.

2.1.7.3. Implantation of intraventricular cannulae.

The intraventricular cannulae were made by attaching a 5cm length of polythene tubing (ref: 800/100/140/100, Portex Ltd.) to an 8mm length of stainless steel tubing, external diameter 0.5mm. The system was filled with artificial cerebrospinal fluid of the following composition (mM): NaCl 127.65, KCl 2.55, $CaCl_2.2H_2O$ 1.26, $MgCl_2.6H_2O$ 0.93, $NaHCO_3$ 23.7, NaH_2PO_4 1.51, glucose 3.38. This is a modification of that used by Merlis (1940). The end of the polythene tubing was then heat-sealed.

The cannula was mounted in the carrier of the stereotaxic frame. The incisor bar was adjusted to 5.0 mm above the interaural line, since in these experiments the atlas of Pellegrino and Cushman was used. This allows co-ordinates to be measured with reference to the skull landmark bregma

(the point at which the coronal suture crosses the sagittal suture).

Two days following implantation of arterial and venous cannulae, the rats were again anaesthetised with a Hypnorm/Hypnovel mixture such that each received 2.5mg midazolam hydrochloride, 5mg fluanisone and 0.158mg fentanyl citrate per Kg body weight. When unconscious, the rats were immediately placed on a heated blanket and the head centralised in the stereotaxic instrument using the ear and incisor bars. The skull was exposed by a midline incision extending about 20 mm back from the eyes and by scraping away the underlying tissue. The tip of the cannula was located directly over the bregma landmark, and the lateral co-ordinate was noted. The tip of the cannula was then moved -1.8 mm on the lateral plane and a hole drilled in the skull at this point, using a model drill. The tip of the cannula was lowered into the hole until it touched the surface of the brain, the horizontal plane reading was then taken and the tip of the cannula was located in the cerebral ventricle by lowering 3.2 mm (see figure 7).

The cannula was fixed in position by applying a small amount of methylmethacrylate polymer cement (How Medica Ltd.) to the skull and cannula surface. Bonding to the skull was facilitated by prior cleaning of the surface with

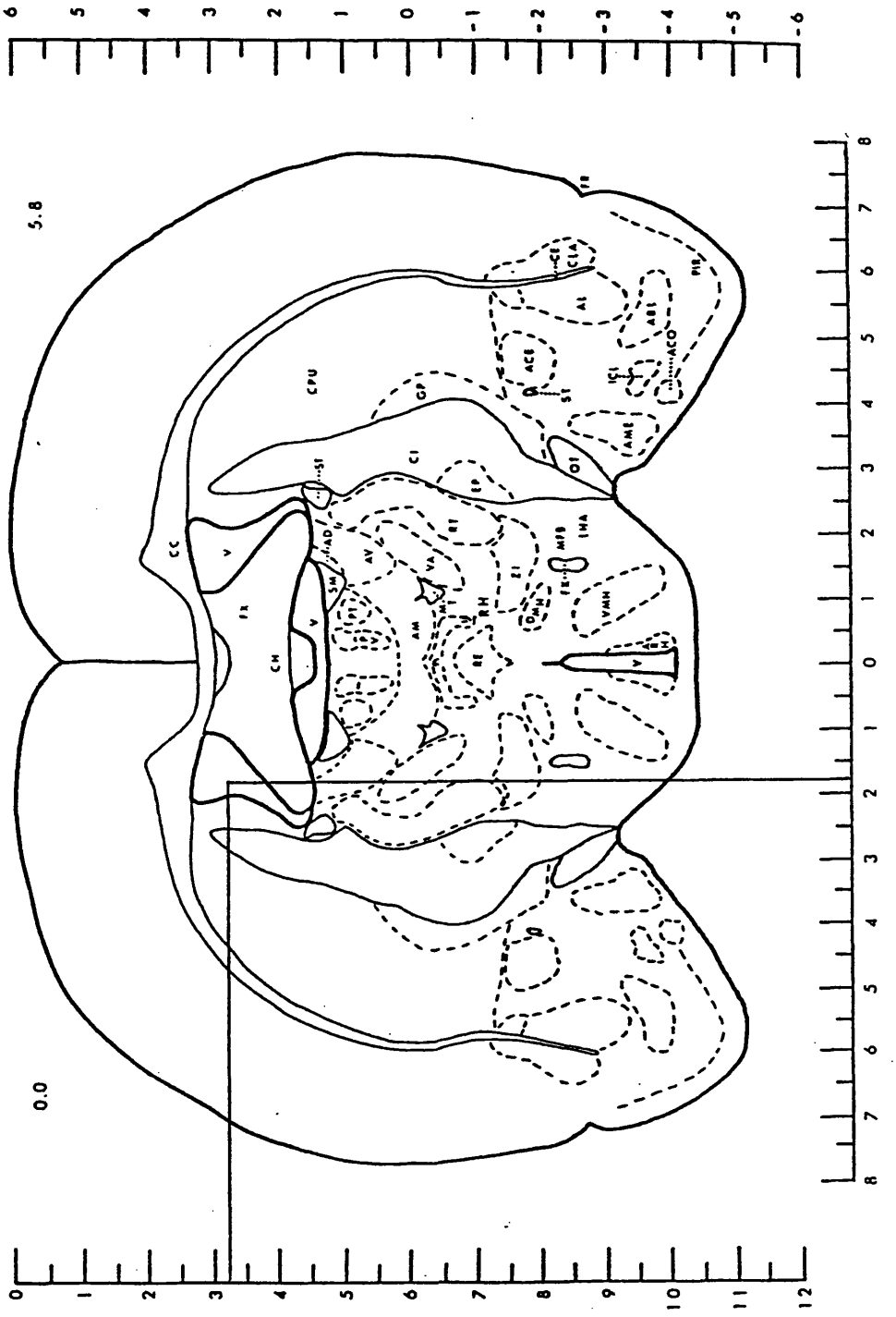

Figure 7. Site of injection into the cerebral ventricle of conscious New Zealand rats.
(Reproduced without permission from Pellegrino and Cushman, 1967)

acetone. The skin incision was then closed with surgical silk sutures and the animals left to recover on a heated blanket for 24 hours.

2.1.7.4. Measurement of blood pressure and heart rate in the conscious rat, and injection of drugs.

One or two days following implantation of the intracerebroventricular cannula, when the animals were completely recovered, they were removed from their home cage and placed in a smaller cage for the duration of the experiment. The movement of the rats was not restricted within the cage but no food or water was provided at this stage.

The intraarterial cannula was connected via an 8 mm length of thin bore stainless steel tubing to a length of polythene tubing (ref: 200/300/030, Portex Ltd.). This, in turn, was connected to a physiological pressure transducer (Gould) and blood pressure and heart rate were measured as described in section 2.1.1.

Injections into the cerebral ventricle were made by means of a 100 microlitre Hamilton syringe, the needle of which fitted directly into the polythene tubing of the cannula.

2.2. DOSING SCHEDULES.

2.2.1. General Considerations.

All drugs for central injection were dissolved in artificial cerebrospinal fluid. All drugs for intravenous injection were dissolved in normal saline. Dosing schedules were the same for all animals, irrespective of strain, type of anaesthetic or whether anaesthetised or not. Specific doses in individual experiments are given in the appropriate section of the results. These are expressed as their salts unless otherwise stated.

2.2.2. Effect of icv pretreatment with beta- adrenoceptor blocking drugs on the response to icv adrenoceptor agonists.

The dose of adrenoceptor agonist was contained in 5 microlitre (mcl) artificial csf and was injected at 2 mcl/minute. Where appropriate, the dose of beta-adrenoceptor blocking drug was dissolved in 10 mcl artificial csf and also injected at 2 mcl/minute, commencing 15 minutes before injection of the adrenoceptor agonist. When more than one drug was injected into the cerebral ventricle, the cannula was removed from the animal between injections and thoroughly washed through with

artificial csf before being loaded with the next solution and relocated in the cerebral ventricle.

2.2.3. Effect of iv beta- adrenoceptor blocking drug pretreatment on the response to icv adrenoceptor agonists.

The dose of adrenoceptor agonist was dissolved in 5 mcl artificial csf and injected at a rate of 2 mcl/minute. Where appropriate, the dose of beta- adrenoceptor blocking drug was dissolved in 0.5 ml saline and was injected at a rate of 0.1 ml/minute starting 15 minutes before the injection of the adrenoceptor agonist. The hole in the skull to facilitate icv injection was drilled before starting the iv injection.

2.2.4. Effect of chronic oral dosing with propranolol on the responses to icv isoprenaline.

The dose of propranolol was given as a single oral dose daily for 14 days in a volume of 5 ml distilled water/Kg body weight. On the day of the experiment, the final dose was given 1 hour before injection of anaesthetic. Isoprenaline was dissolved in 5 mcl artificial csf and injected at 2 mcl/minute.

2.2.5. Effect of icv beta- adrenoceptor blocking drug on the responses to intrahypothalamic adrenoceptor agonist.

The dose of adrenoceptor agonist was dissolved in 1 mcl artificial csf and injected at 0.4 mcl/minute. The dose of beta- adrenoceptor blocking drug was dissolved in 10 mcl artificial csf and injected at 2 mcl/minute, starting 15 minutes before injection of the agonist. Both holes were drilled in the skull directly over the injection sites before any injections were made. The cannula was thoroughly washed with artificial csf between injections.

2.2.6. The effect of chronic oral dosing of beta-adrenoceptor blocking drugs on the responses to intrahypothalamic injection of adrenoceptor agonists.

The dose of beta- adrenoceptor blocking drug was given as a single oral dose daily in a volume of distilled water 5ml/Kg body weight. On the day of the experiment, the final dose was given 1 hour before injection of the anaesthetic. The dose of adrenoceptor agonist was dissolved in 1 mcl artificial csf and injected at 2 mcl/minute. In some animals, icv injection of propranolol preceding injection of the adrenoceptor agonist was made.

2.3. Severance of vagus nerves and spinal cord transection at the C2 level.

Male New Zealand rats, weighing 180 - 230 g, were anaesthetised with Hypnorm/Hypnovel and cannulated as described in section 2.1.1. Prior to mounting the head of the animal on the stereotaxic apparatus, the vagus nerve running alongside the carotid artery was cut on both sides. The spinal cord was transected at the C2 level. Icv injections were then carried out as in section 2.2.2.

2.4. Pretreatment with 6-hydroxydopamine and its effect on icv injections of propranolol and xamoterol.

Male New Zealand normotensive rats, weighing 180 - 230 g, were anaesthetised with ether and injected with 60 mg/Kg 6-hydroxydopamine into the tail vein. 24 hours later, these animals were given icv injections of propranolol and xamoterol as described in section 2.2.2.

2.5. Evaluation of leakage of drugs from the brain following icv injection.

Radiolabelled isoprenaline or propranolol were injected into the cerebral ventricle of the rat and subsequent counting of radioactivity in various organs allowed evaluation of the amount of drug present.

2.5.1. Principles of liquid scintillation counting.

The liquid scintillation counter used was a 1215 Rackbeta II (LKB Wallac).

There are a number of factors which contribute to a count value obtained with a liquid scintillation counter:

1. Counts from radioactivity in the sample.

2. Counts due to background radiation (cosmic rays,etc.)

3. Loss of counts due to the limited efficiency of the light collection system and photomultiplier tube (p.m.t.)

4. Loss of counts due to quenching (chemical or colour) in the scintillator.

5. Counts due to chemiluminescence in the scintillator.

6. Counts due to "noise" in the detection system i.e. thermal noise in the p.m.t.

In order to calculate disintegrations per minute (d.p.m.) from counts per minute (c.p.m.), the effects due to factors 2 - 6 must be removed from the measured c.p.m.

In the case of the Rackbeta, these factors are handled in the following manner:

Two p.m.t.'s are used in coincidence which eliminates most of the effects due to background, chemiluminescence and noise.

Efficiency and degree of quenching are measured for known standards and then appropriate corrections applied to the values obtained for unknown samples. For a standard sample the c.p.m. measured Ni is related to the d.p.m. A via the efficiency Ei.

$$\text{Thus} \quad Ei\% = \frac{Ni}{A} \times 100$$

where i indicates the channel or energy window in which the measurement is made.

Efficiency values were obtained for different degrees of quenching by using a previously prepared series of 10 quenched samples. The result is a curve of efficiency versus degree of quenching. The Rackbeta uses the spline function to fit a curve to the standard points, providing a method of fitting the points without having to assume any particular functional relationship between the efficiency

and quenching. This procedure is performed automatically by the microcomputer which controls the Rackbeta and the programme is stored in the memory of the machine. The d.p.m. value for an unknown sample is then obtained by first measuring the sample c.p.m. and the channels ratio. Then using the channels ratio and the quench curve the value of efficiency is calculated. Finally the d.p.m. is calculated from:

$$A = \frac{Ni}{Ei\%} \times 100$$

2.5.2. Construction of the quench curve.

Ten ml Optiphase 'Safe', the liquid scintillant, was added to each of 10 vials. Optiphase 'Safe' is a multipurpose liquid scintillation cocktail suitable for use with both aqueous and non-aqueous samples (LKB Scintillation Products). To each vial was added a standard volume of the radioactive material such that the total number of counts in the unquenched vial would approximate to the maximum expected from the subsequent studies, The first vial was capped without the addition of any quenching agent.

From preliminary studies, it was found that whole blood was likely to produce the greatest amount of quenching and consequently increasing amounts of untreated blood were

added to the vials according to the following series: 5, 10, 15, 20, 30, 50, 75, 100 and 150 mcl. Following addition of the whole blood, the vials were capped and the samples loaded into the scintillation counter for quench calibration. The counts in each vial were measured and the quench calibration was constructed and stored in the memory.

A fresh calibration was constructed for each isotope used.

2.5.3. Radioisotopes used in the study.

For studies using isoprenaline, the radioisotope used was dl- [7-^{3}H] isoprenaline hydrochloride, specific activity 48 mCi/mg, radioactive concentration 1.0 mCi/ml (Amersham International P.L.C.). A concentrated solution of 'cold', i.e. unlabelled, isoprenaline was added to the radioactive solution to achieve the desired final amount of isoprenaline/ml solution for icv injection.

For studies using propranolol, two separate isotopes were used. Blood content was measured using dl-[4-^{3}H] propranolol hydrochloride, specific activity 67 mCi/mg, radioactive concentration 1 mCi/ml (Amersham International P.L.C.). Tissue content was measured using dl-[^{14}C] propranolol hydrochloride, specific activity 38.4 microCi/mg supplied as solid from ICI P.L.C. The reason

for this difference was that the ^{14}C propranolol had too low a specific activity to be detected in whole blood in the amounts present.

The required amount of 'cold' propranolol was added to the solution of tritiated propranolol, whereas the ^{14}C labelled propranolol was simply dissolved in artificial csf to the required concentration.

2.5.4. Collection of samples for radioactivity measurement.

Male New Zealand normotensive rats, weighing 180 - 230 g, were anaesthetised with a mixture of Hypnorm/Hypnovel and surgically prepared as in section 2.1.1.

Following icv injection of the radiolabelled solution, 0.2 ml of blood was removed from the animal every 2 minutes via the intra-arterial cannula. At a given time following icv injection, the animal was killed by cervical dislocation and the following organs were removed: brain, heart, lungs, liver, kidney.

Clean vials were filled with 10 ml Optiphase 'Safe'. One hundred mcl aliquots of whole blood were added to the vials and thoroughly mixed. The amount of radioactivity in the samples was counted by the Rackbeta liquid scintillation

counter with reference to the pre-constructed quench calibration curve.

The organs removed from the body were weighed and then each was homogenised in 7 ml 0.32 M sucrose solution. One hundred mcl aliquots of the homogenate were added to vials containing Optiphase 'Safe' and the radioactivity in the samples was counted. From the amount of radioactivity in these aliquots, the amount present in the whole organs and total blood volume could then be calculated.

These studies were also carried out in conscious animals; no serial blood analysis was made but analysis of tissue content was carried according to the above method. A specified time after icv injection, rats were decapitated, a small blood sample collected, and the organs removed.

2.6. Dose response curve to intravenous isoprenaline.

Male New Zealand normotensive rats, weighing 180 - 230 g, were dosed daily with a single oral dose of propranolol 60 mg/Kg in distilled water. A dose - response curve to intravenous isoprenaline was constructed for three groups of animals:

1. Control rats which received distilled water for 14 days.

2. Rats given 60 mg/Kg propranolol daily for 14 days, the final dose given 1 hour before anaesthesia.

3. Rats given 60 mg/Kg propranolol daily for 14 days, the final dose being given on the day before experiment.

Animals were anaesthetised with Hypnorm/Hypnovel and the carotid artery and jugular vein were cannulated for measurement of heart rate and injection of isoprenaline respectively (Section 2.1.1.). Serial concentrations of isoprenaline were made in normal saline, the maximum volume injected for a single dose being no greater than 0.4 ml.

Following each injection of isoprenaline, the increase in heart rate was noted, and this was allowed to return to the baseline value before another dose was injected. The dose of isoprenaline was doubled each time until a maximum response was recorded.

2.7. Non - invasive recording of systolic blood pressure using the tail - cuff method.

The tail - cuff method allows measurement of blood pressure in the tail artery of the rat employing non - invasive techniques.

Rats were placed in a heated chamber and maintained at

38 +/- 2°C for 10 minutes. These were then removed from the chamber and a tail cuff and pneumatic pulse transducer (Narco Biosystems Inc.) were applied to the tail. The tail cuff was then inflated by a programmed electrosphygmomanometer (PE - 300, Narco Biosystems Inc.) up to a pressure of 200 mmHg and then deflated back to zero. The pneumatic pulse transducer monitored the pulse pressure, which was recorded on a flat bed recorder (JJ Instruments). The systolic blood pressure of the animal was taken as that pressure at which the pulse was seen to disappear and a flat trace obtained. In order to minimise errors due to any movement of the rat during recording, the blood pressure was measured 3 times over a 10 minute period. The animals were then returned to their home cage for 24 hours.

The next day, the same rats were anaesthetised with Hypnorm/Hypnovel mixture such that each received 5 mg midazolam hydrochloride, 10 mg fluanisone and 0.315 mg fentanyl citrate per Kg body weight. The systolic blood pressure was then monitored whilst the rats were anaesthetised. This gave some indication of the degree of depression of blood pressure caused by anaesthesia.

2.8. Histological techniques for verification of injection site of central injections.

Although the stereotaxic technique allows accurate and reproducible cannula placements, there may be some variation between animals and this requires that placements are verified following experimentation.

2.8.1. Verification of icv injection site.

Five mcl of a 1% w/v aqueous solution of Evans Blue dye was injected into the injection site. The animal was kept alive for five minutes, and during this time, 10 mg of sodium pentobarbitone (Sagatal, May and Baker) was injected i.p. to deepen anaesthesia.

The animal was removed from the stereotaxic instrument and the chest was opened. The descending aorta was clamped behind the left lung. A blunted 19G needle was inserted through the left ventricle and up into the ascending aorta where it was clamped in position. The right ventricle was cut to allow escape of blood and fluid from the system. Forty ml of 10% formol saline (9 g NaCl + 100 ml 40% formaldehyde, made up to 1 litre with distilled water) was injected into the left ventricle. The brain was then

removed from the skull and verification of successful icv injection performed by gross dissection.

For verification in conscious animals, these were anaesthetised with Hypnorm/Hypnovel prior to injection of the dye, and the procedure outlined above carried out.

Verification of icv injections in conscious and anaesthetised animals was carried out after every fifth experiment. Figures 8 and 9 are photographs of brain sections from anaesthetised and conscious rats following injection of dye into the cerebral ventricle.

2.8.2. Verification of injections into the hypothalamus.

Since the target for injection into the hypothalamus was much smaller, it was deemed necessary to verify the injection site in every other animal. One mcl of dye was injected following experimentation, and the brain removed as in section 2.8.1.

Following removal from the skull, the brains were stored in 10% formol saline for at least 7 days. Sections of 24 micron thickness were cut on a freezing microtome from a block of brain containing the injection site. Sections were examined by low-power microscopy and compared with the appropriate saggital section in the stereotaxic atlas.

Figure 8. Injection of Evans Blue dye to indicate site of injection into the lateral cerebral ventricle of anaesthetised rats. (See also figure 2, this chapter)

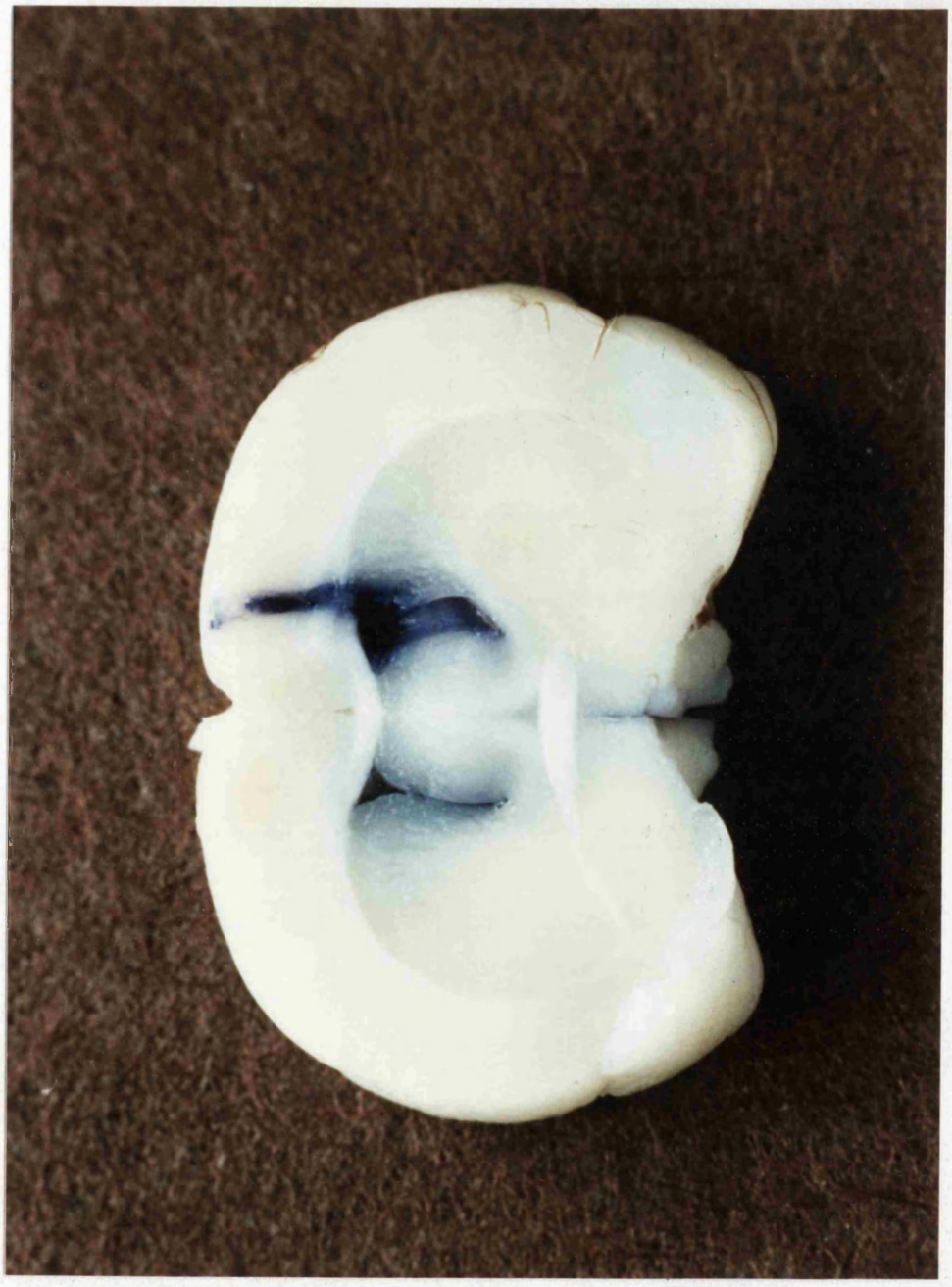

Figure 9. Injection of Evans Blue dye to indicate site of injection into the cerebral ventricle of conscious New Zealand normotensive rats. (See also figure 7, this chapter)

2.9. Data analysis.

Mean arterial pressure was calculated from the blood pressure trace using the following formula:

$$MAP = \frac{\text{systolic bp} - \text{diastolic bp}}{3} + \text{diastolic bp}$$

Data were analysed for statistical significance by Students' t-test for unpaired comparisons.

2.10. Materials used in this study.

	Supplier
Noradrenaline hydrochloride	Sigma
Adrenaline bitartrate	Sigma
Isoprenaline hydrochloride	Sigma
Atenolol *	ICI PLC
Propranolol hydrochloride	Sigma
ICI 118,551 hydrochloride *	ICI PLC
Clenbuterol hydrochloride	Dr. Karl Thomae
Xamoterol fumarate *	ICI PLC
Sodium thiobutobarbitone (Inactin) *	BYK
Sodium pentobarbitone (Sagatal)	May & Baker
Midazolam hydrochloride (Hypnovel)	Roche
Fluanisone/fentanyl citrate (Hypnorm)	Janssen
Sodium heparin	Sigma
Formaldehyde solution	BDH Chemicals
^{14}C-propranolol *	ICI PLC
3H-propranolol	Amersham Intnl.
3H-isoprenaline	Amersham Intnl.
Optiphase 'Safe'	LKB Scint. Prod.
6-hydroxydopamine	Sigma

* These substances kindly donated by ICI PLC

icv injected drugs dissolved in artificial csf
iv injected drugs dissolved in 0.9% NaCl

Chapter 3.

RESULTS AND DISCUSSION

GENERAL CONSIDERATIONS.

This chapter has been divided into sections, each dealing with a series of experiments related by site of injection, strain of rat or presence of anaesthesia.

Each section has three parts, the first a description of the experiments and their results, the second, graphical representation of the results and the third a discussion of the results in that section. A general discussion will be given at the end of the chapter.

For ease of cross-reference, at the end of most headings in this chapter, the section in the Materials and Methods chapter describing the dosing schedule has been indicated in parentheses.

Group data are expressed as mean +/- standard error of the mean.

Graphs involving injection of adrenoceptor agonists and pretreatment with beta- adrenoceptor antagonists commence at the beginning of the injection of adrenoceptor agonist.

The group size is presented in parentheses in the graph key and this is followed by the mean starting blood pressure and heart rate values for the group immediately before injection

of adrenoceptor agonist.

3.1. Measurement of systolic blood pressure by the tail cuff method. (2.7.)

The resting systolic blood pressure of a representative sample of New Zealand normotensive rats was found to be 143 +/- 3 mmHg, n = 28. Similarly, that of a representative sample of New Zealand hypertensive rats was found to be 156 +/- 3 mmHg, n = 27. Following injection of Hypnorm/Hypnovel, the systolic blood pressures were 124 +/- 5 mmHg and 152 +/- 7 mmHg respectively.

Measurement of systolic blood pressure of unrestrained rats by the tail cuff method revealed two important facts:

1. The systolic blood pressure in unanaesthetised rats was not significantly different between 'hypertensive' and 'normotensive' animals.

When the strain was described by Phelan (1968), the average blood pressure for hypertensive males was 175 mmHg, that of the normotensive males 115 mmHg. It was clear that the colony of New Zealand rats held at Bath University was no longer divisible into hypertensive and normotensive animals. The reason for this may be that matings had been carried out between animals with unrecorded blood pressures over several

generations. This particular strain of genetically hypertensive rat had been initially achieved by successive mating of rats with above average blood pressure, the increase in each generation being approximately 2 mmHg for the first 20 generations of inbreeding (Phelan and Simpson, 1987). It is feasible that matings between rats with unrecorded blood pressure could lead to a loss of hypertension in the colony.

At the beginning of this study, the normotensive animals from the New Zealand colony were used as an alternative model of normotension to repeat some of the work which had previously used Wistar rats. After much work had been done using these animals, some experiments were repeated using the New Zealand hypertensive animals. It was at this time that the loss of hypertension in the colony was discovered. This necessitated the use of Japanese Okamoto spontaneously hypertensive rats as the model of hypertension with Wistar Kyoto rats as normotensive controls. Since only the normotensive rats from the New Zealand colony were used in this study, they have been referred to in this chapter as simply 'New Zealand rats'.

2. The anaesthesia produced by Hypnorm/Hypnovel did not produce an excessive fall in blood pressure.

The falls in systolic blood pressure caused by anaesthesia were 19 and 4 mmHg for 'normotensive' and 'hypertensive' rats respectively. It is possible this could be a result of the

absence of stress in the anaesthetised animal when the systolic blood pressure was recorded.

It was decided that the combination of Hypnorm and Hypnovel would be suitable as an anaesthetic in this strain since it produced good surgical anaesthesia, could be administered by a single intraperitoneal injection and appeared to have minimal effect on resting blood pressure.

3.2. Intracerebroventricular (icv) injection of beta-adrenoceptor agonists in the anaesthetised New Zealand rat and the effect of pretreatment with beta- adrenoceptor blocking agents.

3.2.1. Icv injection of beta- adrenoceptor blocking agents.

Icv injection of 30 micrograms (mcg) propranolol produced a large fall in heart rate, the maximum of 44 beats per minute (bpm) occurring 10 minutes after start of injection. This was accompanied by a slight increase in mean arterial pressure of 15 mmHg.

Atenolol (30 mcg) injected icv had little effect on either heart rate or blood pressure, the maximum fall in heart rate was 8 bpm at 15 minutes with an accompanying change in mean arterial pressure of 3-4 mmHg.

ICI 118,551 (30 mcg icv) produced a tachycardia of 22 bpm at 15 minutes and a biphasic change in mean arterial pressure ranging from -8 to +8 mmHg. (See figures 1a and 1b).

3.2.2. Intravenous (iv) injection of beta- adrenoceptor blocking agents.

12 mcg propranolol injected iv produced a bradycardia of 94 bpm at 5 minutes with a slight increase of mean arterial

pressure of 15 mmHg.

Atenolol (12 mcg iv) reduced heart rate by a maximum of 132 bpm and mean arterial pressure by 13 mmHg at 15 minutes.

The bradycardia produced by 12 mcg ICI 118,551 was maximal at 5 minutes (24 bpm) and had returned to pre-injection level at 15 minutes. Mean arterial pressure was increased by 25 mmHg over the 15 minute period. (See figures 2a and 2b).

3.2.3. Icv injection of propranolol and adrenaline. (2.2.2.)

Icv injection of 20 mcg adrenaline produced a small increase in mean arterial pressure (maximum 14 mmHg, 4 minutes after injection) which had returned to normal after 10 minutes. This was accompanied by a fall in heart rate of 22 bpm after 10 minutes.

Following previous icv injection of 30 mcg propranolol, icv injection of adrenaline produced a significantly ($p<0.05$) greater increase in mean arterial pressure, the maximum at 4 minutes now being 36 mmHg. The bradycardia produced by icv adrenaline was not significantly altered following pretreatment with propranolol. (See figures 3a and 3b).

3.2.4. Icv injection of clenbuterol following pretreatment with propranolol. (2.2.2. and 2.2.3.)

Icv injection of clenbuterol (5 mcg) produced a fall in mean arterial pressure (maximum 30 mmHg) and tachycardia (maximum 34 bpm). The hypotension was unaffected by pretreatment with 12 mcg propranolol iv, but was significantly ($p<0.01$) reduced by the icv injection of 30 mcg propranolol. The increase in heart rate remained unaffected following pretreatment with propranolol injected either iv or icv. (See figures 4a and 4b).

3.2.5. Pretreatment with propranolol and icv injection of xamoterol. (2.2.2. and 2.2.3.)

Icv injection of xamoterol (5 mcg) produced a fall in blood pressure and an increase in heart rate of 13 mmHg and 33 bpm at 20 minutes respectively (see figures 5a and 5b).

Pretreatment with propranolol, both 12 mcg iv and 30 mcg icv, significantly reversed the hypotension ($p<0.01$ for both groups). 30 mcg propranolol icv did not affect the tachycardia produced by xamoterol, but this response was significantly ($p<0.01$) potentiated following pretreatment with 12 mcg iv.

3.2.6. Effect of treatment with 6-hydroxydopamine on the responses to icv xamoterol. (2.4.)

Pre-dosing animals with 60 mg/Kg 6-hydroxydopamine iv and bilateral vagotomy reduced the hypotensive response to icv injection of xamoterol (5 mcg), but this reduction did not reach statistical significance. The reversal of this hypotension by pretreatment with icv propranolol (30 mcg) was unaffected by 6-hydroxydopamine treatment and bilateral vagotomy. In 6-hydroxydopamine treated animals, pretreatment with icv propranolol significantly reversed the hypotension induced by icv xamoterol. (See figure 6a).

The tachycardia caused by icv xamoterol was significantly reduced in animals treated with 6-hydroxydopamine, but this significance was lost in the group also pretreated with icv propranolol. (See figure 6b).

3.2.7. Effect of pretreatment with beta- adrenoceptor blocking agents on the responses to icv isoprenaline.

Following icv injection of isoprenaline, a dose dependant hypotension was observed, the maximum occurring 6 minutes after start of injection. This was accompanied by tachycardia which was not dose - dependant; heart rate increased rapidly over the first 6 to 8 minutes and then remained at this level over the remainder of the experiment.

The maximum values of hypotension and tachycardia following icv isoprenaline are shown below.

Dose isoprenaline (mcg)	Max. hypotension (mmHg)	Max. tachycardia (bpm)	Figure
1.0	18	60	7a & 7b
5.0	22	32	8a & 8b
20.0	51	67	12a & 12b

3.2.7.1. Pretreatment with propranolol and the responses to 1 mcg isoprenaline icv. (2.2.2. and 2.2.3.)

The hypotension produced by 1 mcg isoprenaline icv was reduced to a similar extent by both 30 mcg propranolol icv and 24 mcg propranolol iv, this reduction only reaching statistical significance at 4 to 8 minutes following start of injection, i.e. at that time at which maximum hypotension was produced by icv isoprenaline (sig. $p<0.05$). However, 60 mcg propranolol icv significantly reversed the hypotension produced by 1 mcg isoprenaline ($p<0.01$), the maximum hypotension being 11 mmHg (see figure 7a).

Pretreatment with 30 mcg propranolol icv and 24 mcg propranolol iv did not significantly affect the tachycardia produced by 1 mcg isoprenaline, although a slight reduction in the increase in heart rate was seen with both pretreatments (see figure 7b). Pretreatment with 60 mcg propranolol icv significantly reduced the tachycardia produced by 1 mcg isoprenaline icv ($p<0.05$), the maximum rise in heart rate being 25 bpm.

3.2.7.2. Icv injection of 5 mcg isoprenaline and the effect of pretreatment with beta- adrenoceptor blocking drugs. (2.2.2., 2.2.3. and 2.2.4.)

The duration of hypotension produced by 5 mcg isoprenaline was significantly reduced ($p<0.05$) by 30 mcg propranolol icv, whereas the maximum value was not significantly attenuated. Pretreatment with 12 mcg propranolol iv had no effect on the hypotensive response to icv isoprenaline (see figure 8a). In animals dosed orally with 60 mg/Kg propranolol daily for 14 days, icv injection of 5 mcg isoprenaline resulted in a marked hypertension, maximum 31.5 mmHg, this was highly significant from the hypotension seen in untreated animals ($p<0.001$).

The tachycardia induced by 5 mcg isoprenaline icv was significantly enhanced following pretreatment with icv and iv propranolol ($p<0.01$). Oral dosed animals also showed an enhanced tachycardia to icv isoprenaline, this only reaching

significance 15 minutes after start of injection ($p<0.05$).

The maximum values for tachycardia are shown below (see figure 8b)

Pretreatment	Max. tachycardia (bpm)
Nil	32
12 mcg propranolol iv	100
30 mcg propranolol icv	84
60 mg/Kg propranolol po	64

The hypotension and tachycardia seen following 5 mcg isoprenaline icv were not significantly altered by a single oral dose of 60 mg/Kg propranolol given one hour before anaesthesia (see figures 9a and 9b).

Pretreatment with atenolol, 30 mcg icv and 12 mcg iv, did not significantly alter the fall in blood pressure produced by 5 mcg isoprenaline icv (see figure 10a). Pretreatment with 30 mcg ICI 118,551 icv significantly reduced the hypotension ($p<0.01$), whereas 12 mcg iv also reduced the hypotension, but

to a lesser extent (see figure 11a).

Pretreatment with ICI 118,551 had little effect on the isoprenaline induced tachycardia; this was also unaffected by icv atenolol but significantly ($p<0.05$) potentiated by iv atenolol 10 minutes after injection of isoprenaline (see figures 11b and 10b respectively).

3.2.7.3. Icv injection of propranolol and 20 mcg isoprenaline. (2.2.2.)

The hypotension and tachycardia produced by 20 mcg isoprenaline icv were not significantly modified by pretreatment with 30 mcg propranolol icv (see figures 12a and 12b), although the degree of hypotension was attenuated by approximately 30%.

3.2.8. Response to icv propranolol and isoprenaline in animals with the vagus nerves and the spinal cord severed. (2.3.)

Following severance of both vagus nerves and the spinal cord at the C_2 level, icv injection of isoprenaline caused a greater degree of hypotension, but this did not reach significance. Tachycardia was essentially unaffected (see figures 13a and 13b).

However, the increase in mean arterial pressure and the fall in heart rate produced by icv propranolol were abolished by severance of the spinal cord and vagus nerves (see figures 14a and 14b).

3.2.9. Dose response curves to iv isoprenaline. (2.6.)

Dose response curves to iv isoprenaline were constructed for control animals and those dosed orally with propranolol. The curves were all shifted to the right for all groups dosed with propranolol. In the groups dosed for 14 days, the maximum increase in heart rate was much greater than in the control group and the group given a single oral dose of propranolol (see figure 15).

The dose of isoprenaline that elicited 50% of the maximum increase in heart rate (ED50) was calculated for each group, and the ratio of the ED50 of each treatment group to the control was calculated - this gives a measure of the number of folds of dose of isoprenaline needed in each treatment group to elicit the same response as obtained in the control group and thus a measure of the amount of beta- adrenoceptor blockade achieved by propranolol. The values are tabulated below.

Treatment group	ED50	Ratio of ED50 with control group
Control	0.2 ng	1
60 mg/Kg propranolol Single dose	125.9 ng	630
60 mg/Kg propranolol x 14 days Last dose 24 hours previously	47.9 ng	240
60 mg/Kg propranolol x 14 days Last dose 1 hour previously	316.2 ng	1585

3.2.10. Leakage of drugs from the brain following icv injection. (2.5.)

Blood levels of isoprenaline measured following icv injection of ^{3}H- isoprenaline showed that after 20 minutes, approximately 1% of the injected dose was present in the blood stream. Figure 16 shows the amount of isoprenaline in nanograms present in the blood stream following icv injection of 1, 5 and 20 mcg isoprenaline. Figure 17 is a measure of that present in the blood stream adjusted per milligram of injected dose, this illustrates that the amount of drug present in the blood is a percentage of that injected

initially. The amount of propranolol measured in the blood also approximates to 1% of the 30 mcg injected icv (see figure 18).

Tissue levels of isoprenaline following icv injection of 5 mcg indicate that over the time of the experiment there is at least 60% of the injected dose remaining in the brain. There are small amounts of isoprenaline present in both the heart and lung, whereas in the liver and kidney, the levels of isoprenaline increase with time (see figure 20).

Following icv injection of propranolol, a large percentage appears to remain in the brain for up to 30 minutes. Significant amounts were found in heart and lung, but extremely small amounts were found in the mesenteric bed. As with isoprenaline, the content of propranolol in liver and kidney increased with time (see figure 19).

The profiles for isoprenaline and propranolol were found to be similar in both blood and organ content.

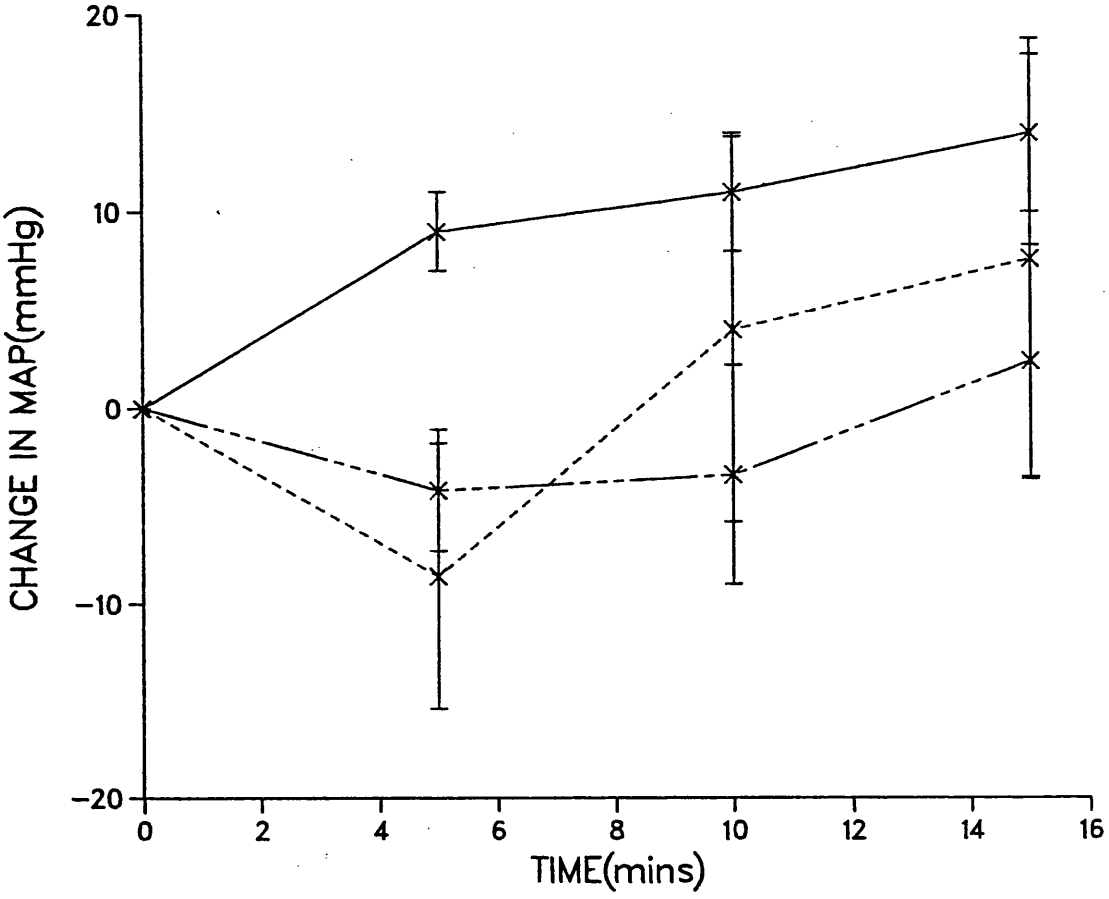

Figure 1a.

Figures 1a and 1b. Change in mean arterial pressure and heart rate following icv injection of beta- adrenoceptor blocking drugs in anaesthetised New Zealand rats.

x———x 30 mcg propranolol (n=6)

x- - - - - - - -x 30 mcg ICI 118,551 (n=6)

x———- - -———x 30 mcg atenolol (n=6)

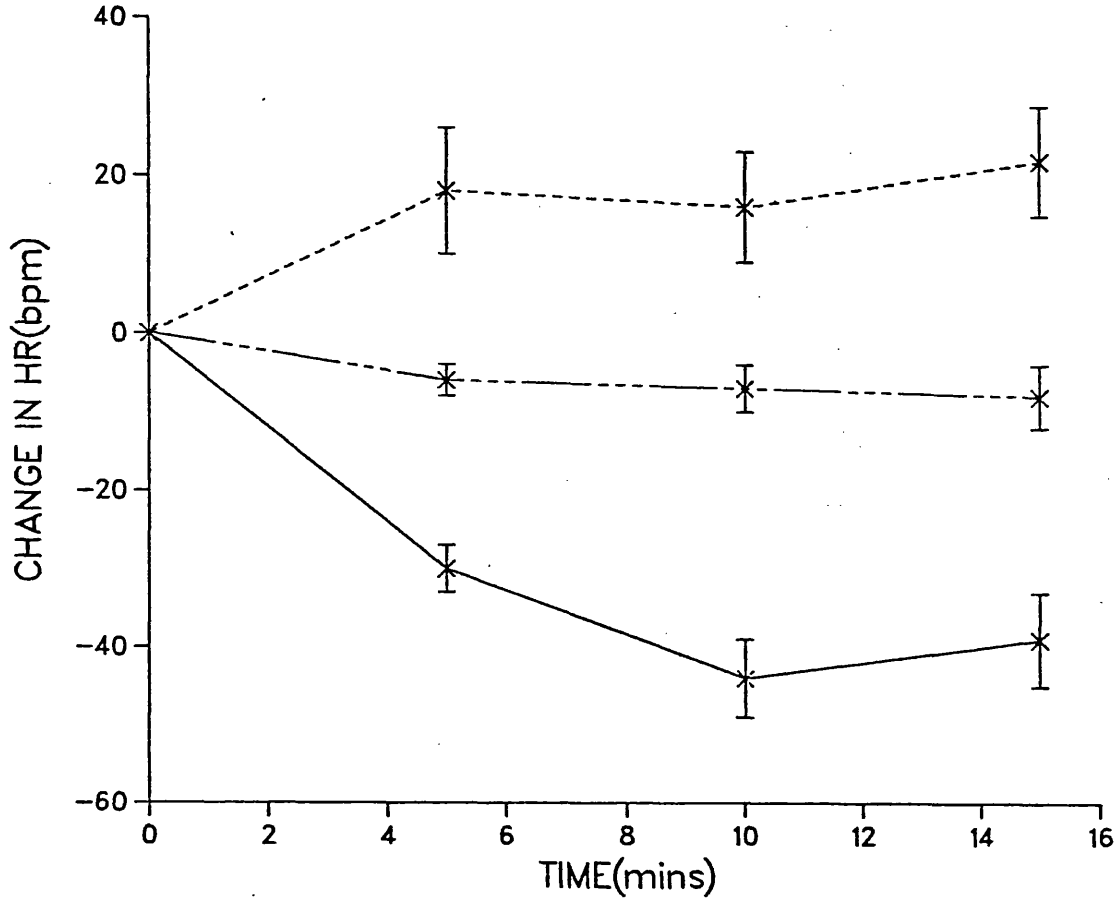

Figure 1b.

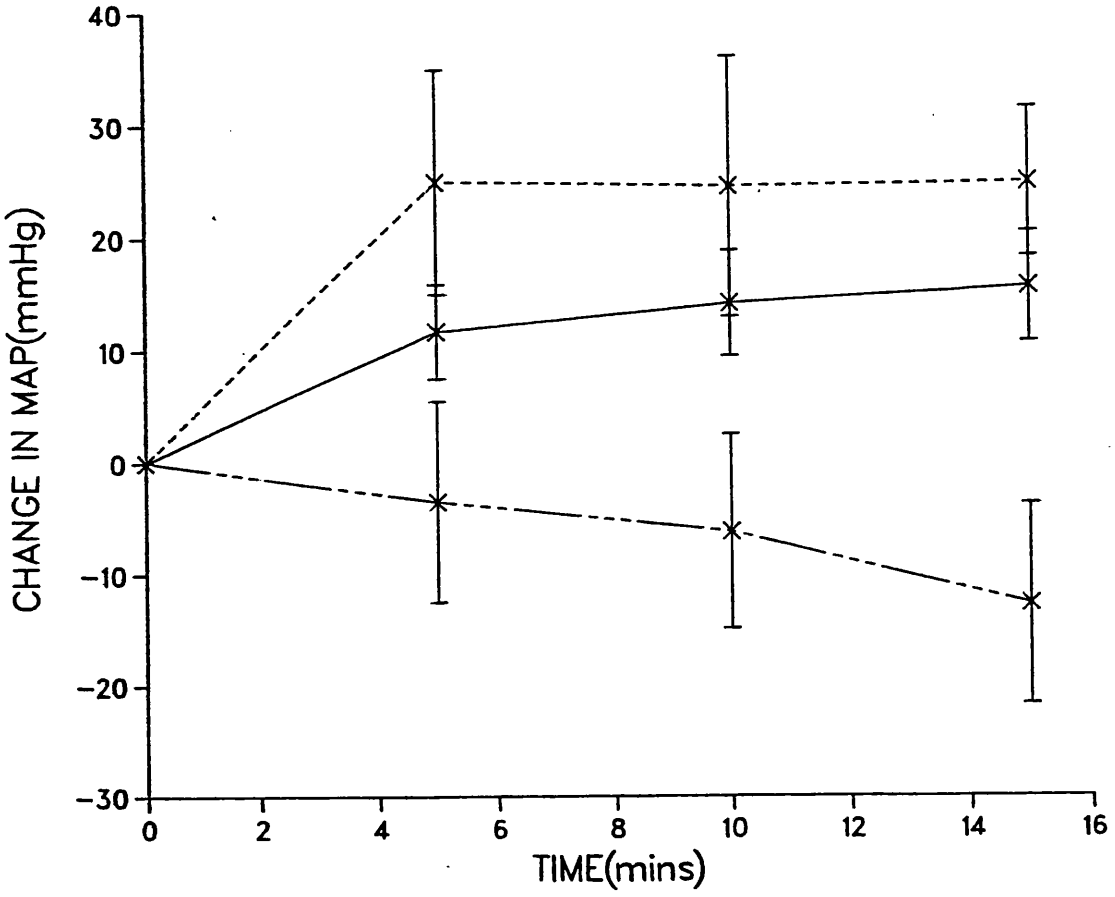

Figure 2a.

Figures 2a and 2b. Change in mean arterial pressure and heart rate following intravenous injection of beta-adrenoceptor blocking drugs in anaesthetised New Zealand rats.

x———x 12 mcg propranolol (n=6)

x- - - - - - -x 12 mcg ICI 118,551 (n=6)

x——- - - -x 12 mcg atenolol (n=6)

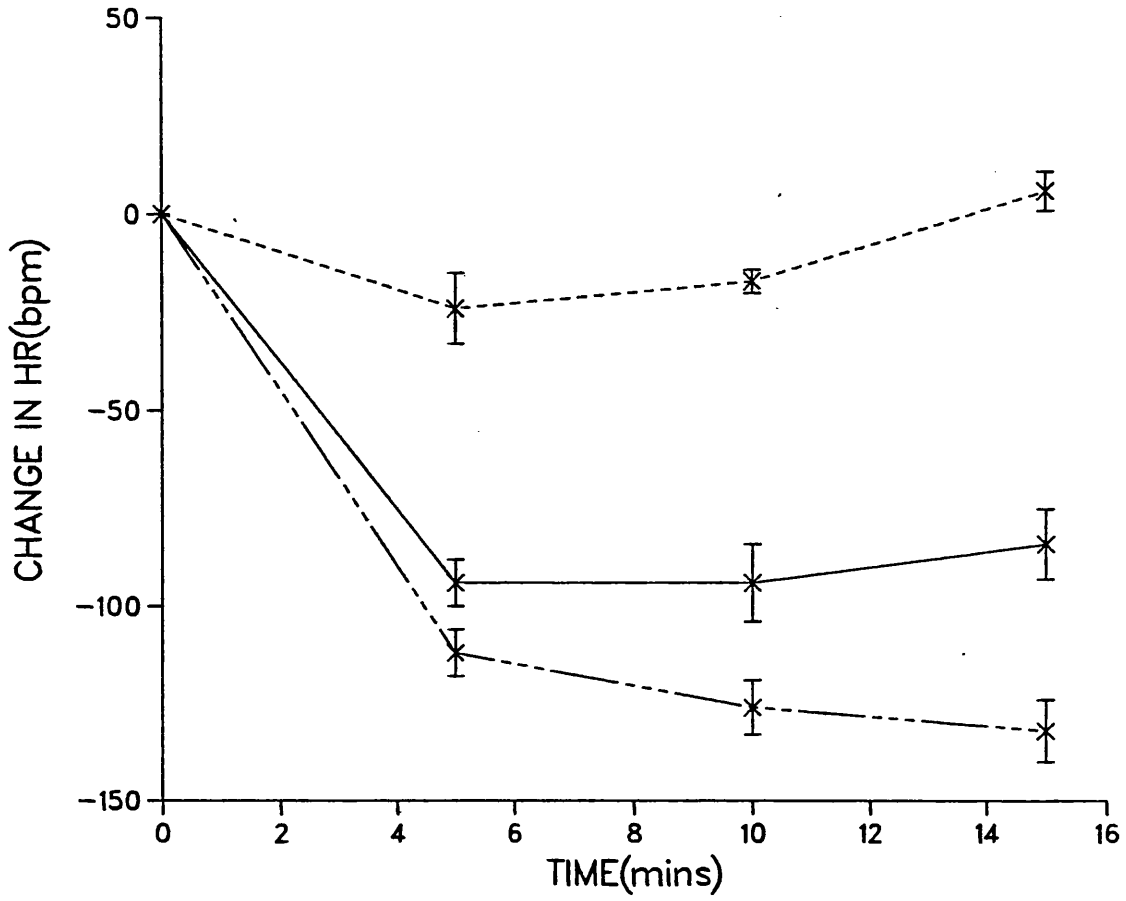

Figure 2b.

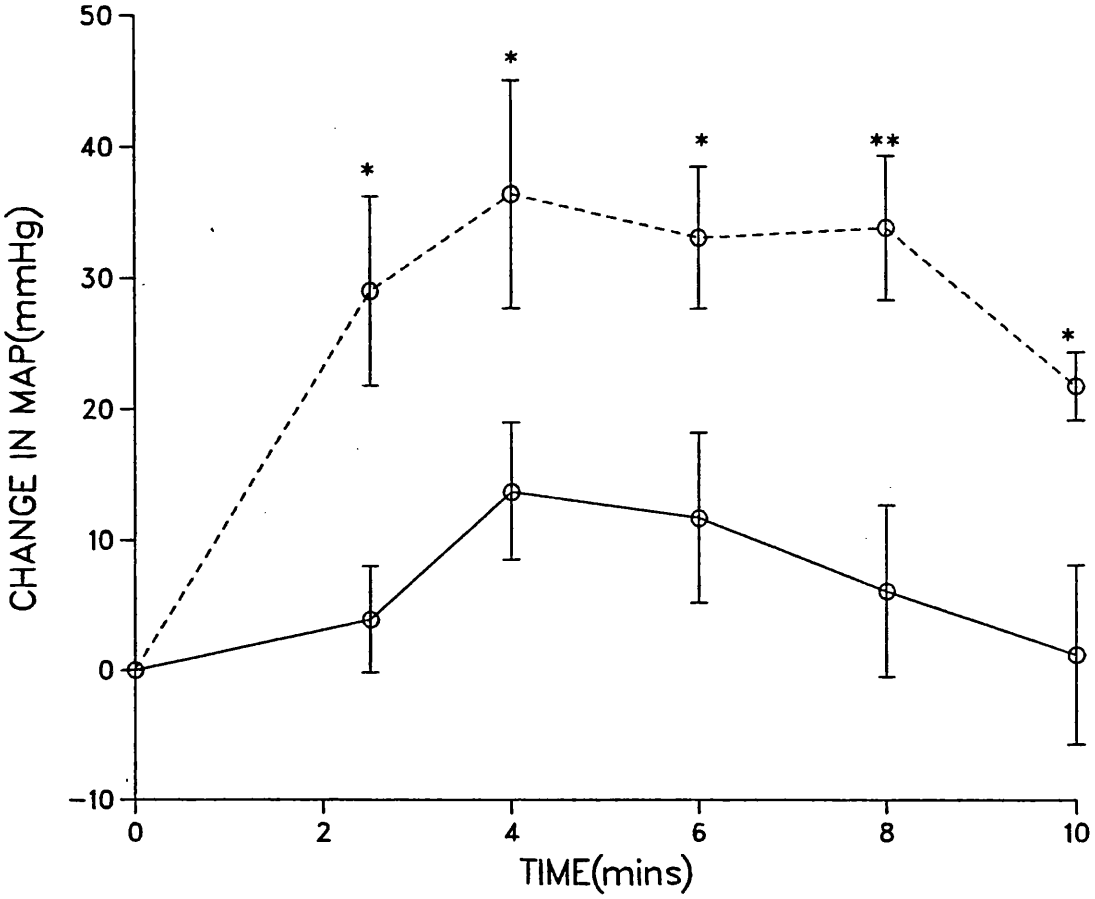

Figure 3a.

Figures 3a and 3b. Change in mean arterial pressure and heart rate in anaesthetised New Zealand rats following icv injection of 20 mcg adrenaline.

⊖————⊖ No pretreatment (n=7) 139 mmHg, 393 bpm

⊖- - - - - -⊖ 30 mcg propranolol icv (n=7) 164 mmHg, 301 bpm

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

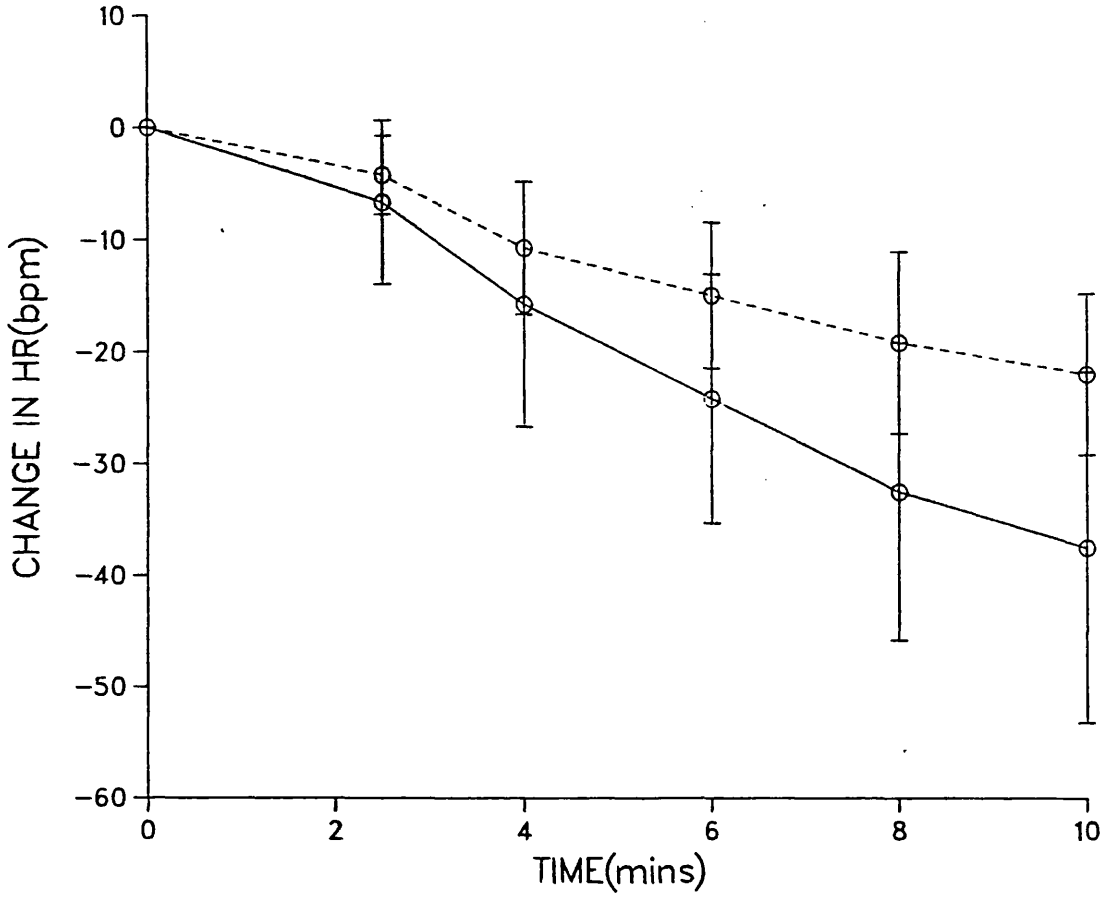

Figure 3b.

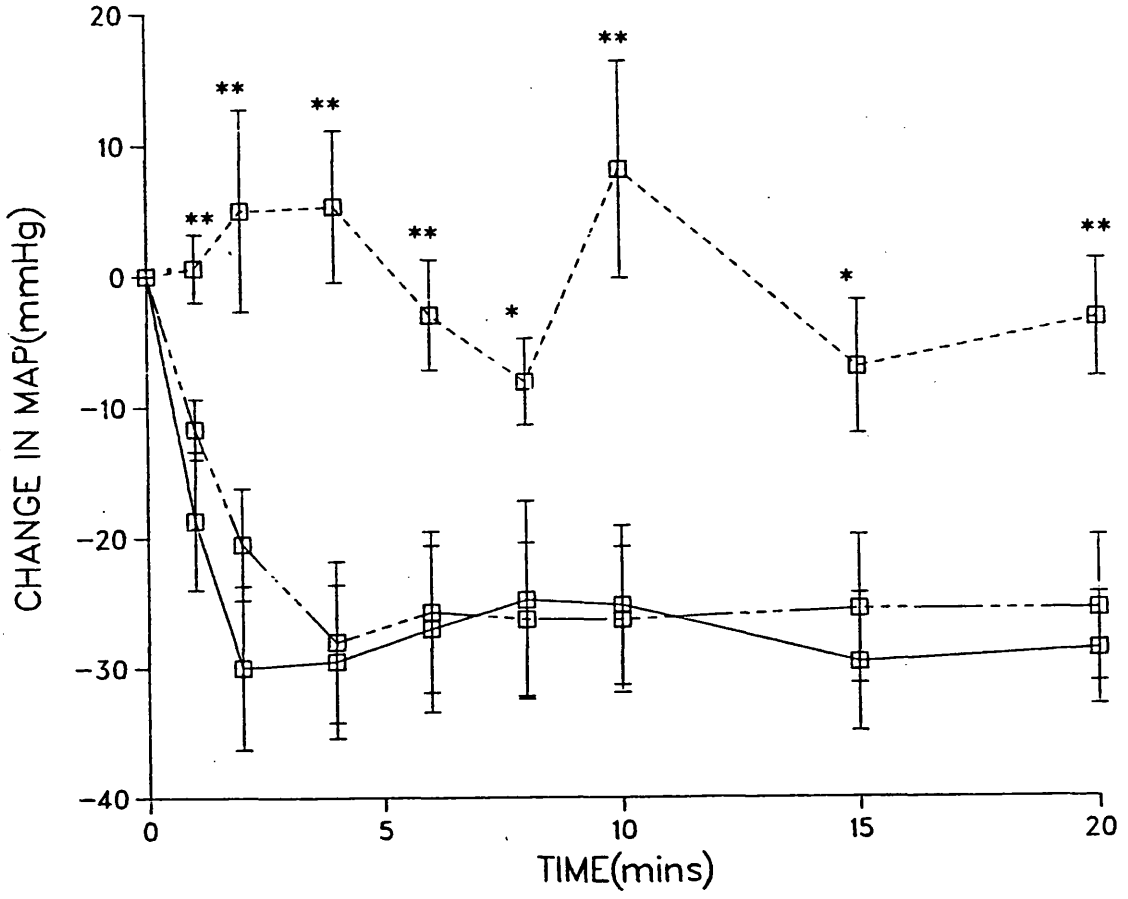

Figure 4a.

Figures 4a and 4b. Change in mean arterial pressure and heart rate produced by 5 mcg clenbuterol icv in anaesthetised New Zealand rats.

——— No pretreatment (n=7) 81 mmHg, 411 bpm.

- - - - - - 30 mcg propranolol icv (n=7) 104 mmHg, 424 bpm.

——- - -—— 12 mcg propranolol iv (n=6) 112 mmHg, 383 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

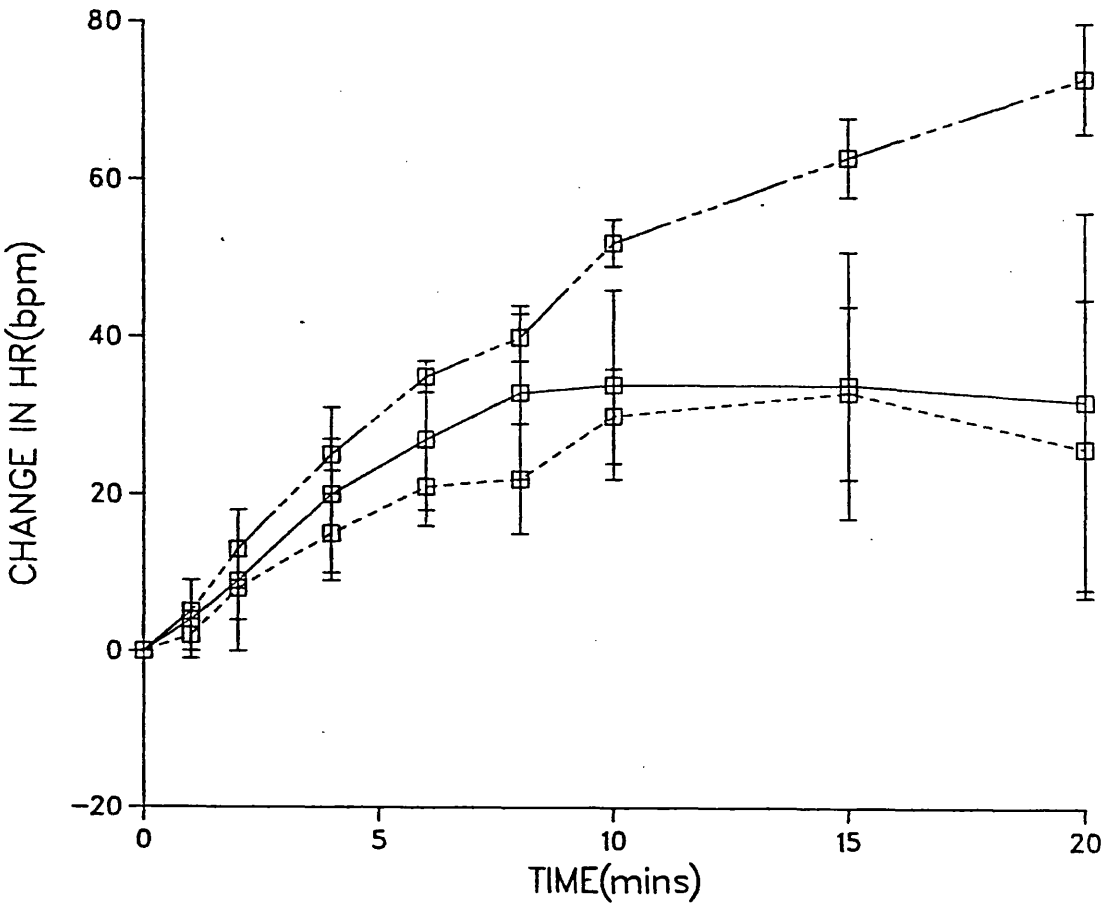

Figure 4b.

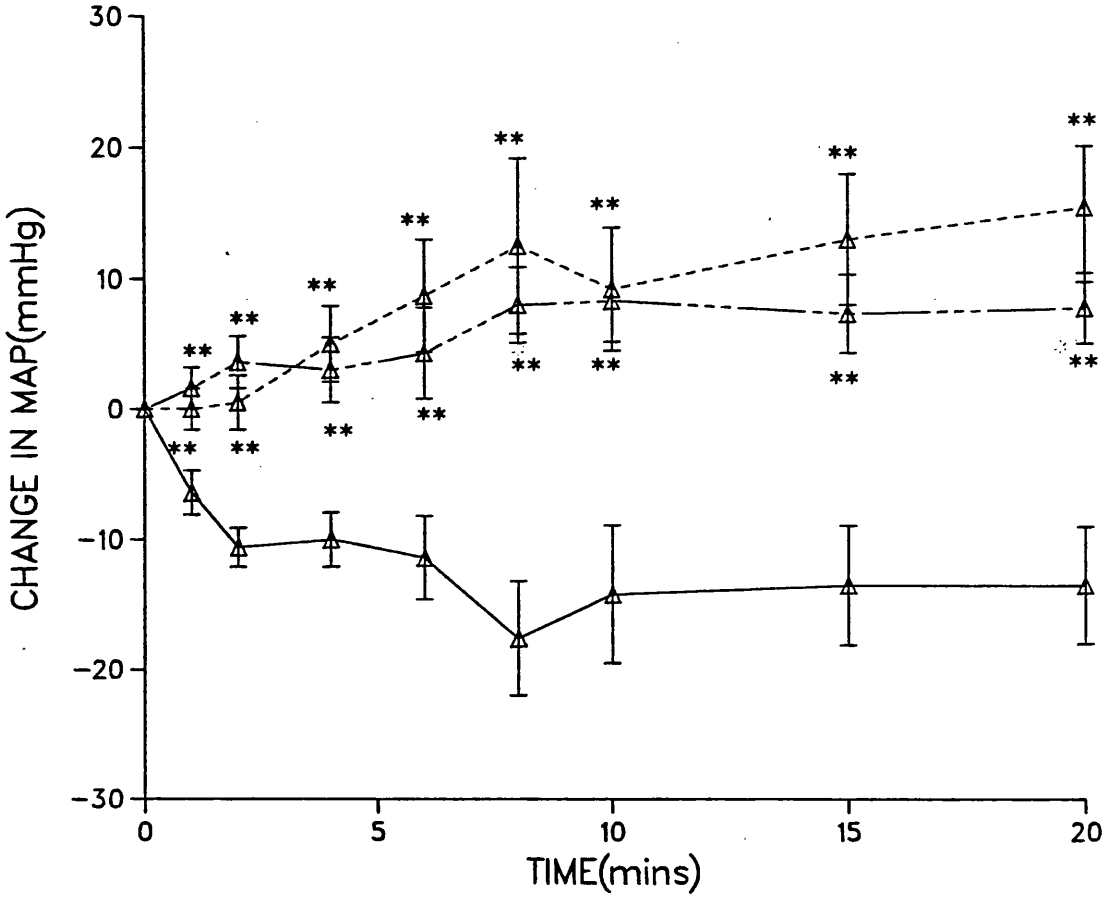

Figure 5a.

Figures 5a and 5b. Change in mean arterial pressure and heart rate following 5 mcg xamoterol icv in anaesthetised New Zealand rats.

△——△ No pretreatment (n=8) 100 mmHg, 431 bpm.

△- - - - - -△ 30 mcg propranolol icv (n=6) 88 mmHg, 377 bpm.

△——···——△ 12 mcg propranolol iv (n=6) 110 mmHg, 382 bpm.

Significant difference from no pretreatment group denoted:

** $p<0.01$

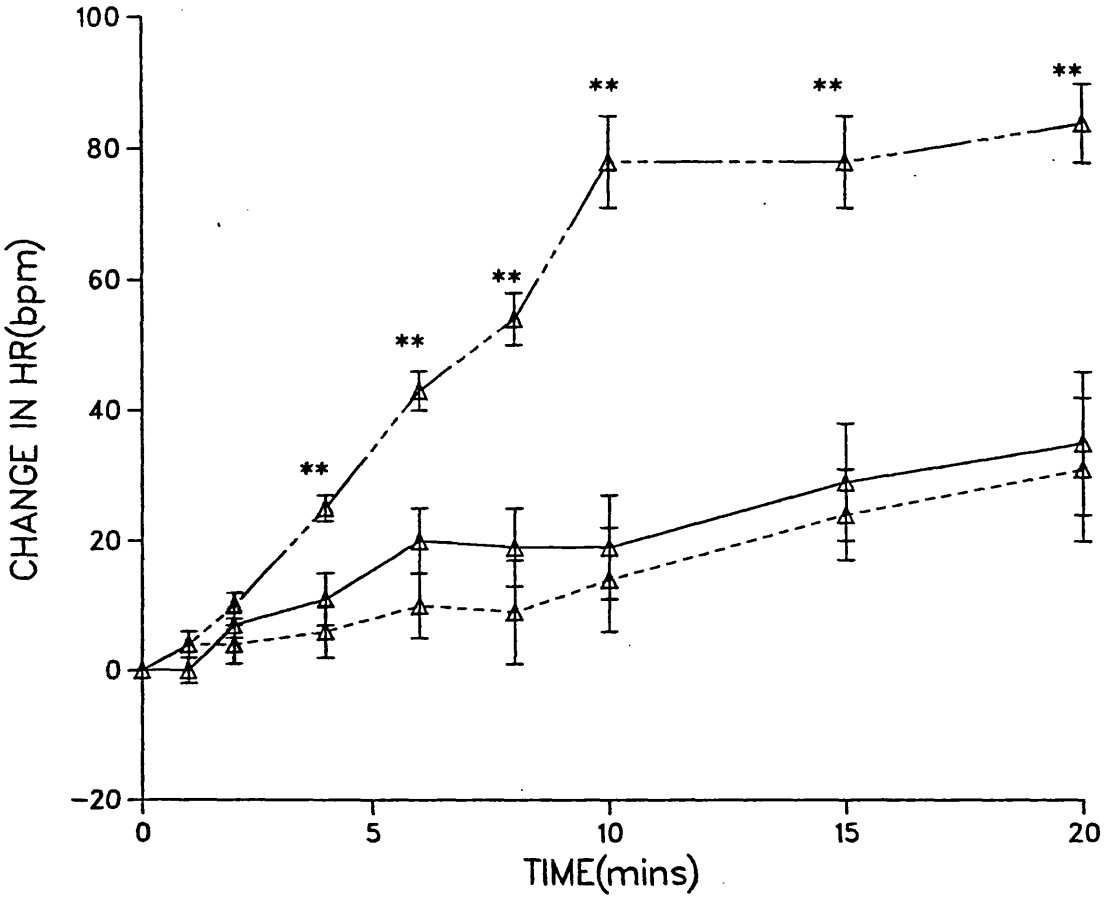

Figure 5b.

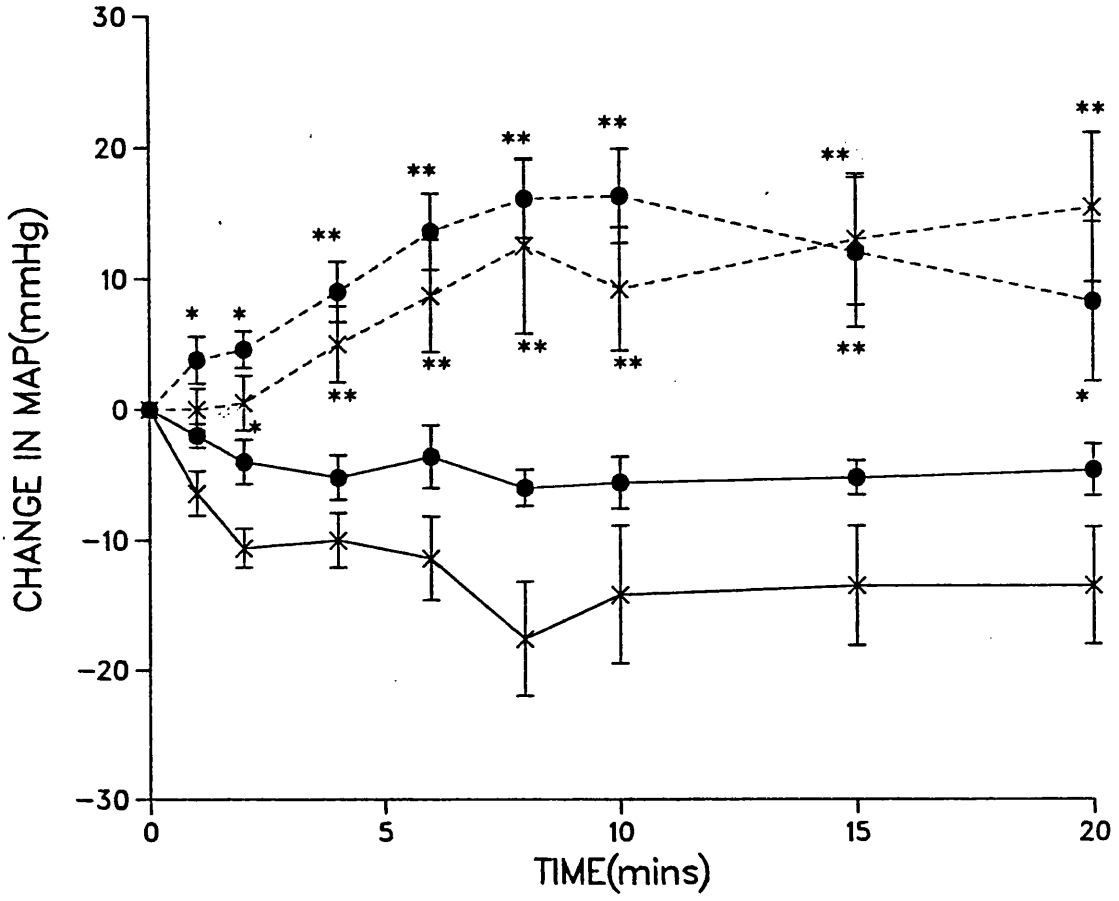

Figure 6a.

Figures 6a and 6b. Change in mean arterial pressure and heart rate following 5 mcg xamoterol icv in anaesthetised New Zealand rats.

x———x No pretreatment (n=8) 100 mmHg, 431 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=6) 88 mmHg, 377 bpm

●———● Animals dosed with 60 mg/Kg 6-hydroxydopamine and bilateral vagotomy No pretreatment (n=6) 69 mmHg, 410 bpm.

●- - - - - - -● Animals dosed with 60 mg/Kg 6-hydroxydopamine and bilateral vagotomy 30 mcg propranolol icv (n=6) 105 mmHg, 405 bpm.

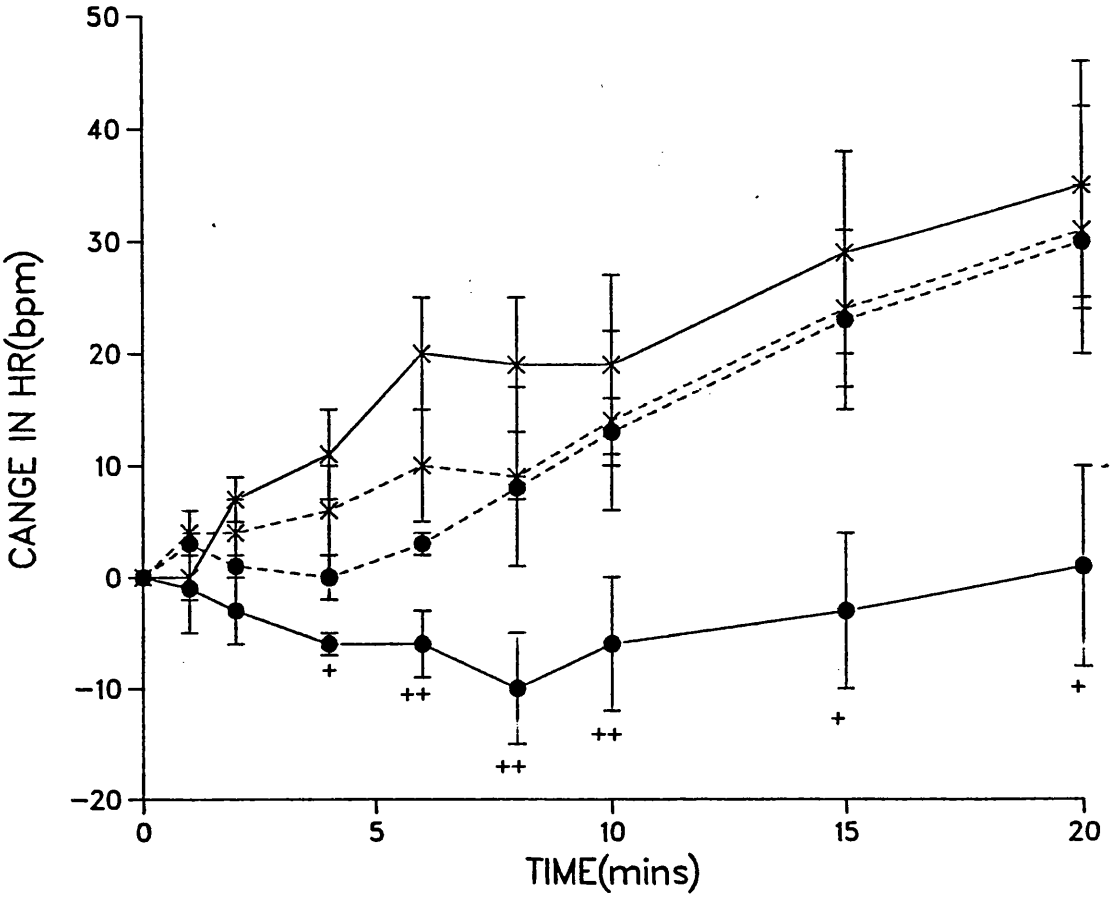

Figure 6b.

Figures 6a and 6b(contd.).

Significant difference between groups denoted:

* p<0.05 ** p<0.01

Differences denoted are between:
x——————x and x- - - - - -x
●——————● and ●- - - - - -●

Significant difference between x——————x and ●——————● denoted:

+ p<0.05 ++ p<0.01

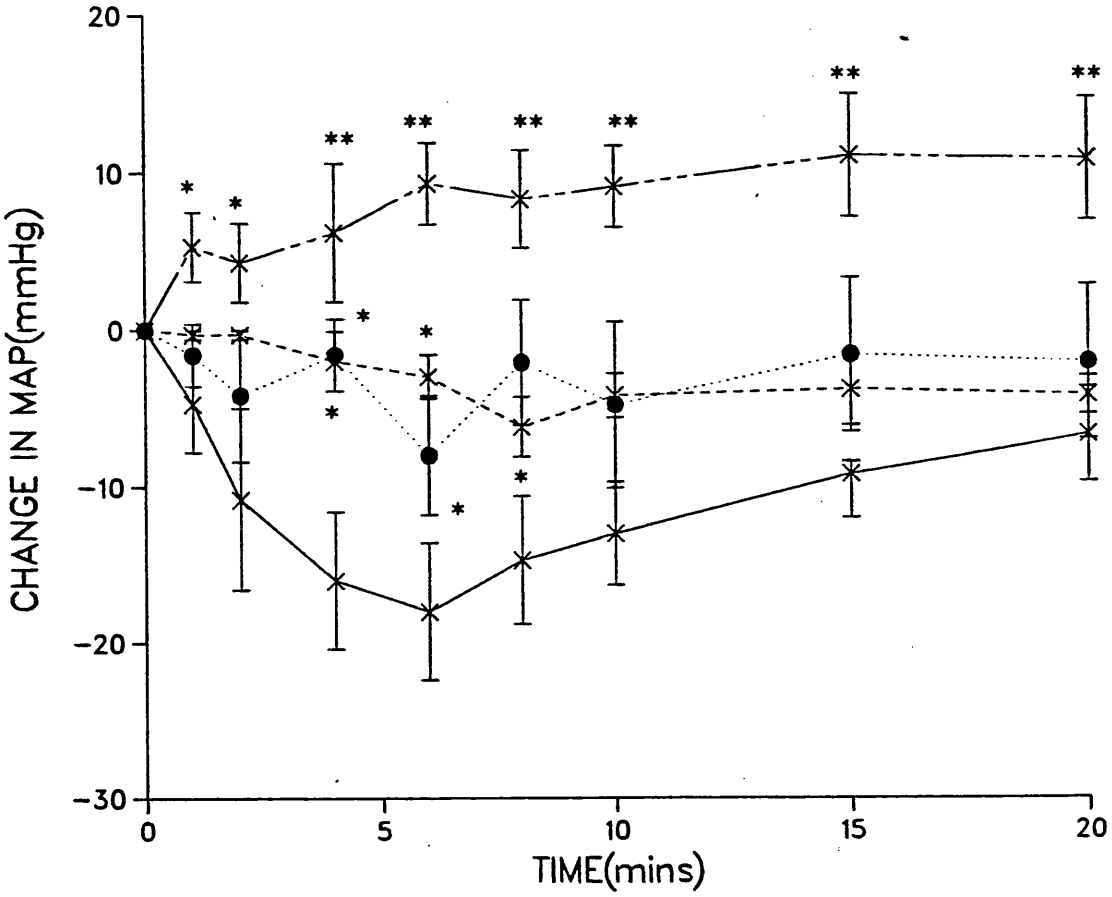

Figure 7a.

Figures 7a and 7b. Change in mean arterial pressure and heart rate produced by 1 mcg isoprenaline icv in anaesthetised New Zealand rats.

x———x No pretreatment (n=6) 99 mmHg, 428 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=6) 94 mmHg, 435 bpm.

x—— - - - ——x 60 mcg propranolol icv (n=6) 111 mmHg, 360 bpm.

●·····● 24 mcg propranolol iv (n=6) 102 mmHg, 304 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

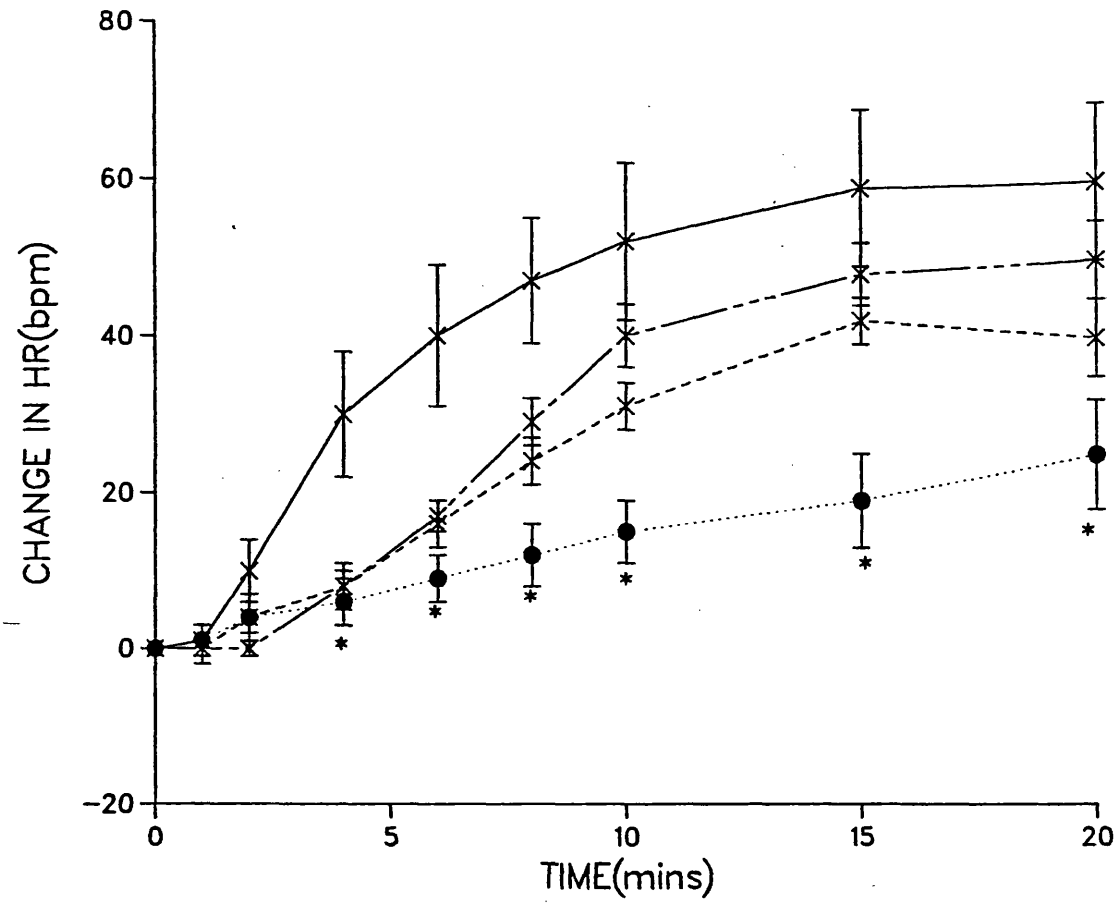

Figure 7b.

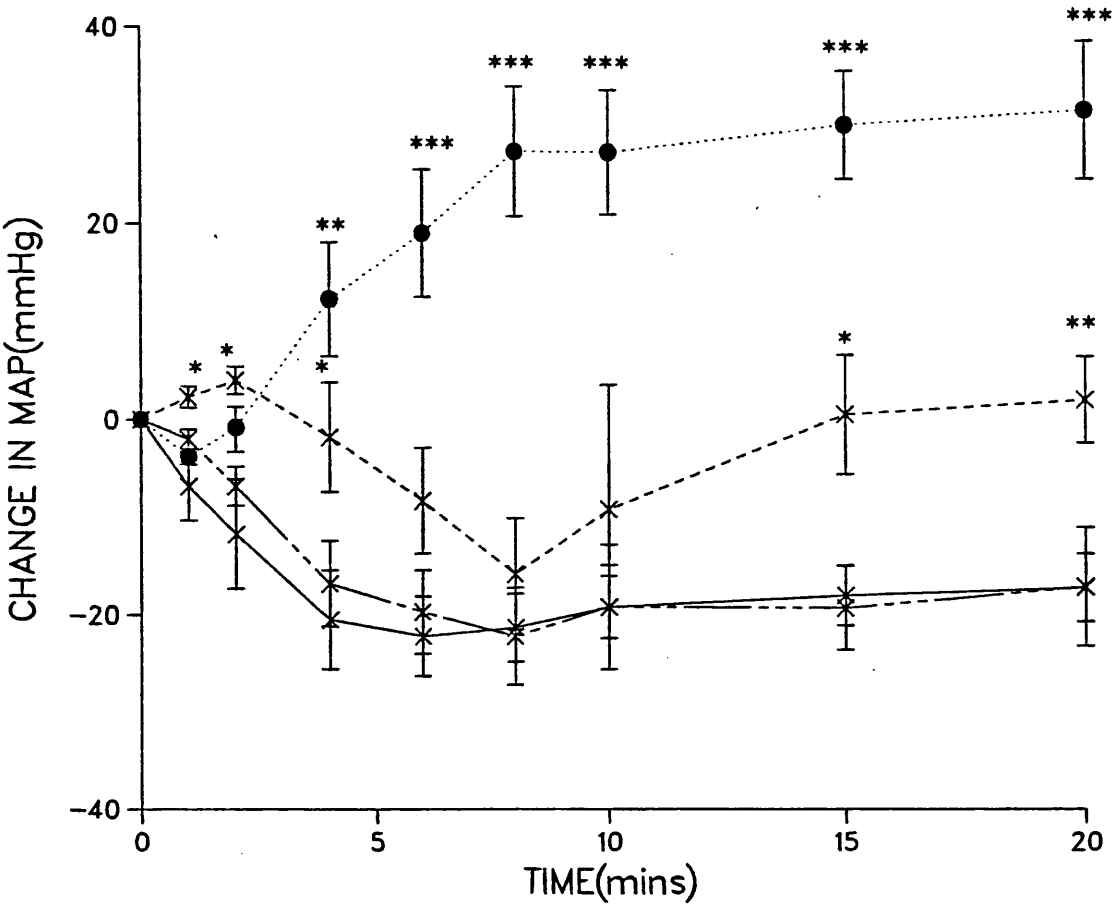

Figure 8a.

Figures 8a and 8b. Change in mean arterial pressure and heart rate produced by 5 mcg isoprenaline icv in anaesthetised New Zealand rats.

x————x No pretreatment (n=6) 87 mmHg, 427 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 88 mmHg, 395 bpm.

x—— - - -—x 12 mcg propranolol iv (n=6) 103 mmHg, 370 bpm.

●·····● 60 mg/Kg propanolol po daily for 14 days (n=6) 124 mmHg, 316 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

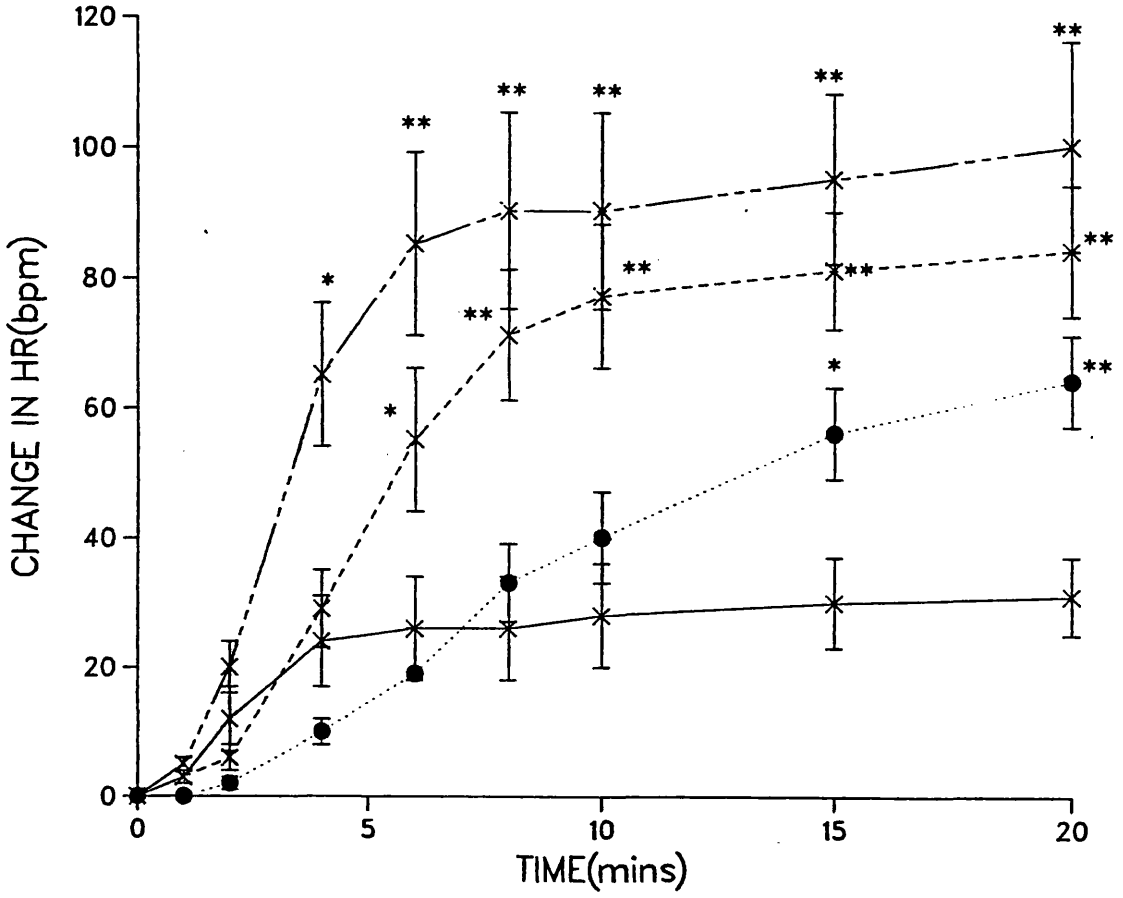

Figure 8b.

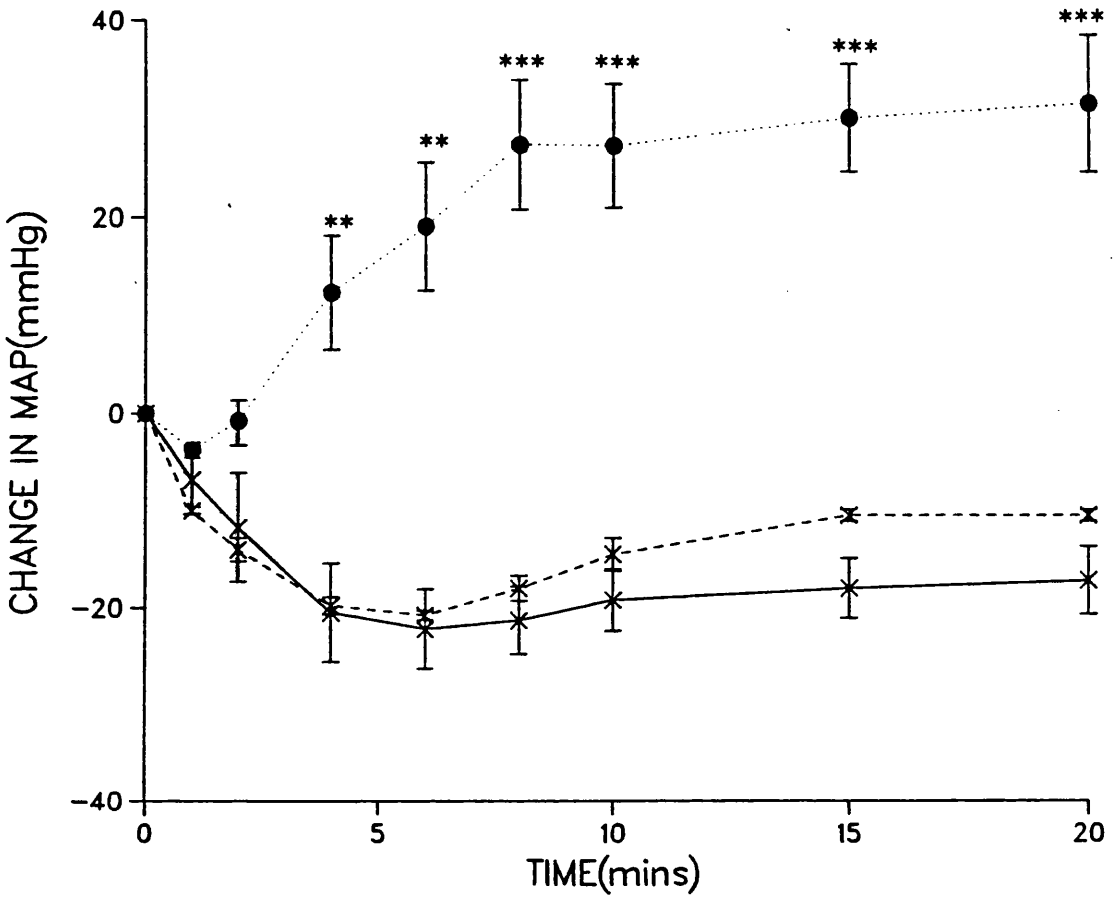

Figure 9a.

Figures 9a and 9b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in anaesthetised New Zealand rats.

x———x No pretreatment (n=6) 87 mmHg, 427 bpm.

x- - - - - - -x 60 mg/Kg propranolol po single dose (n=4) 145 mmHg, 285 bpm.

●·····● 60 mg/Kg propanolol po daily for 14 days (n=6) 124 mmHg, 316 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

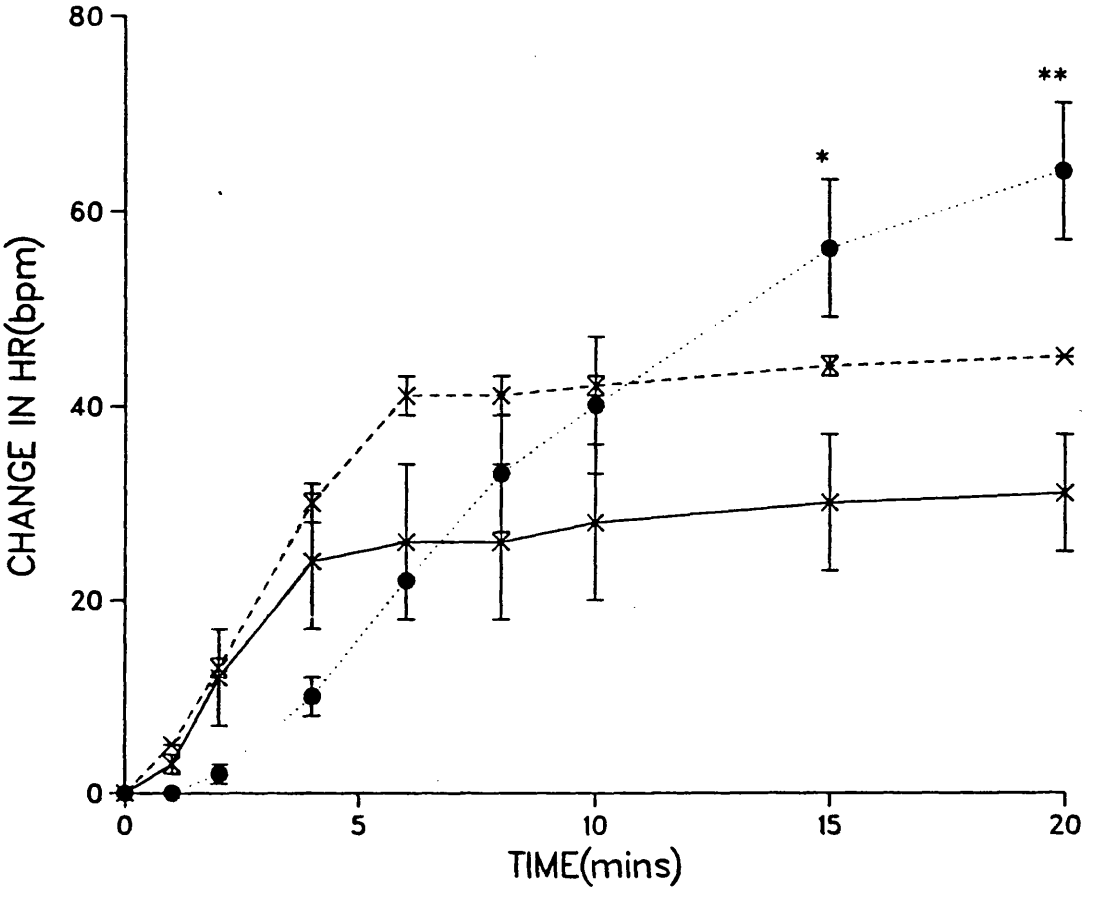

Figure 9b.

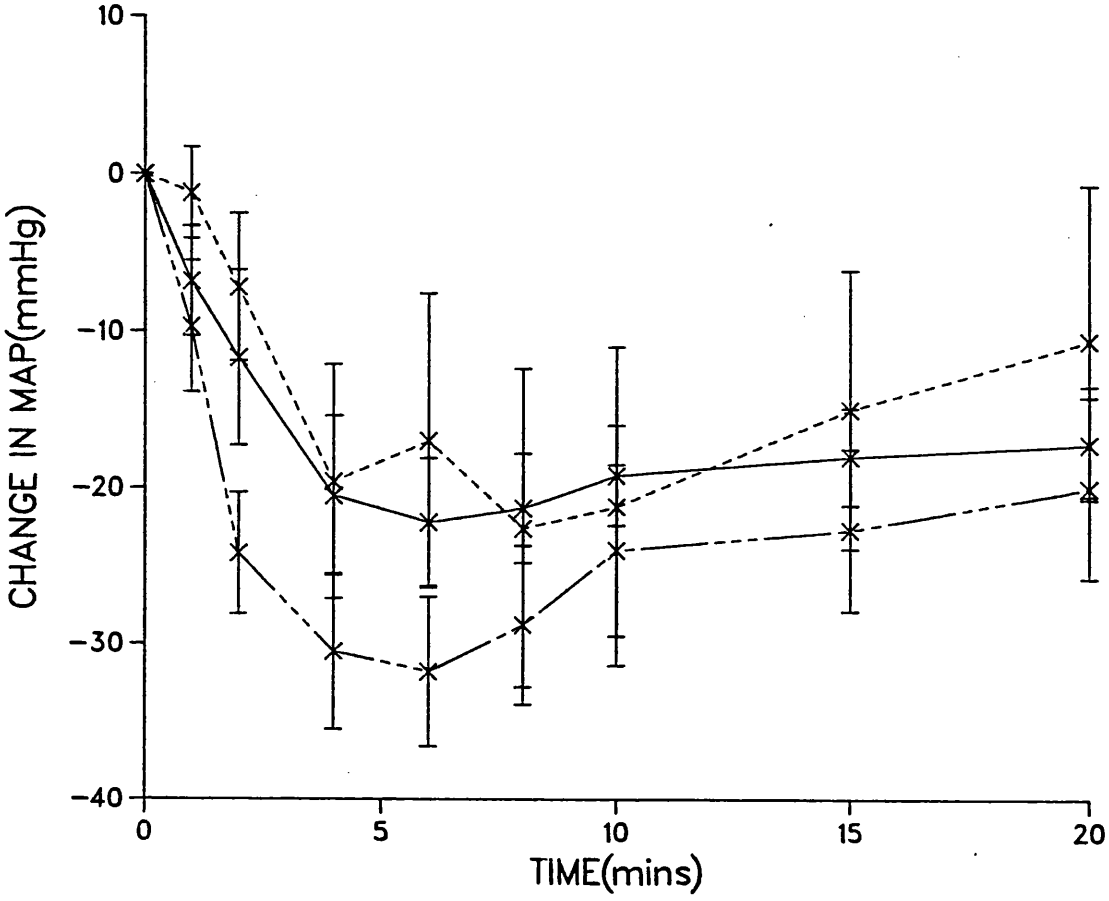

Figure 10a.

Figures 10a and 10b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in anaesthetised New Zealand rats.

x———x No pretreatment (n=6) 87 mmHg, 427 bpm.

x- - - - - -x 30 mcg atenolol icv (n=5) 110 mmHg, 468 bpm.

x——··——x 12 mcg atenolol iv (n=6) 102 mmHg, 333 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$

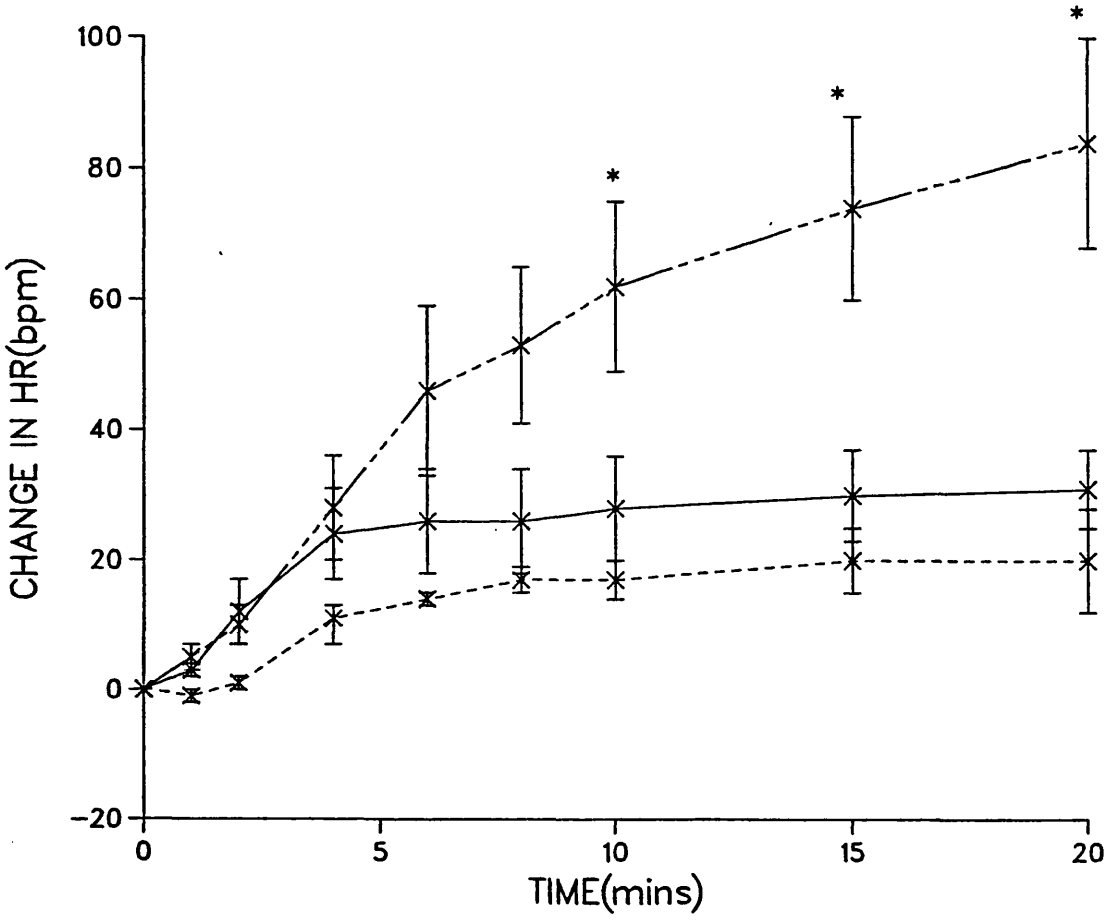

Figure 10b.

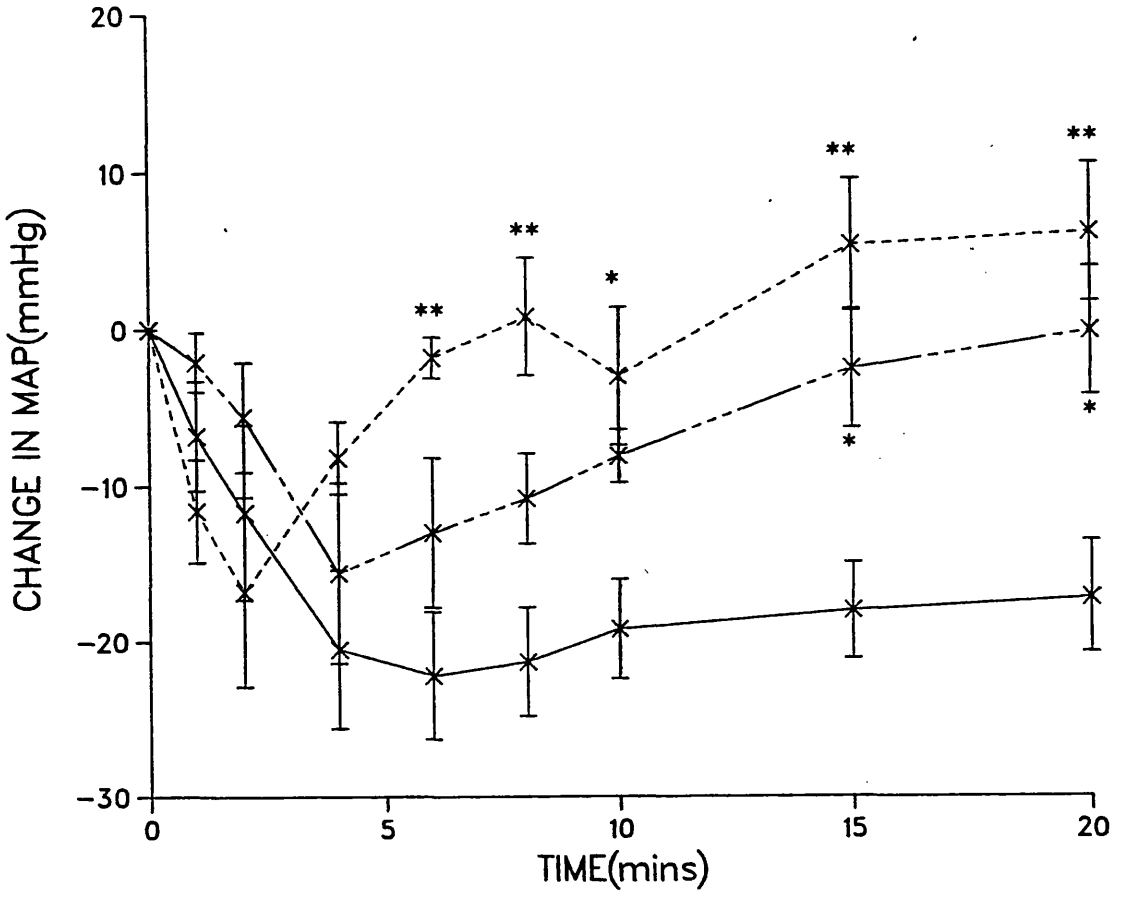

Figure 11a.

Figures 11a and 11b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in anaesthetised New Zealand rats.

x———x No pretreatment (n=6) 87 mmHg, 427 bpm.

x- - - - - -x 30 mcg ICI 118,551 icv (n=5) 112 mmHg, 479 bpm.

x———- - - -x 12 mcg ICI 118,551 iv (n=6) 109 mmHg, 430 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

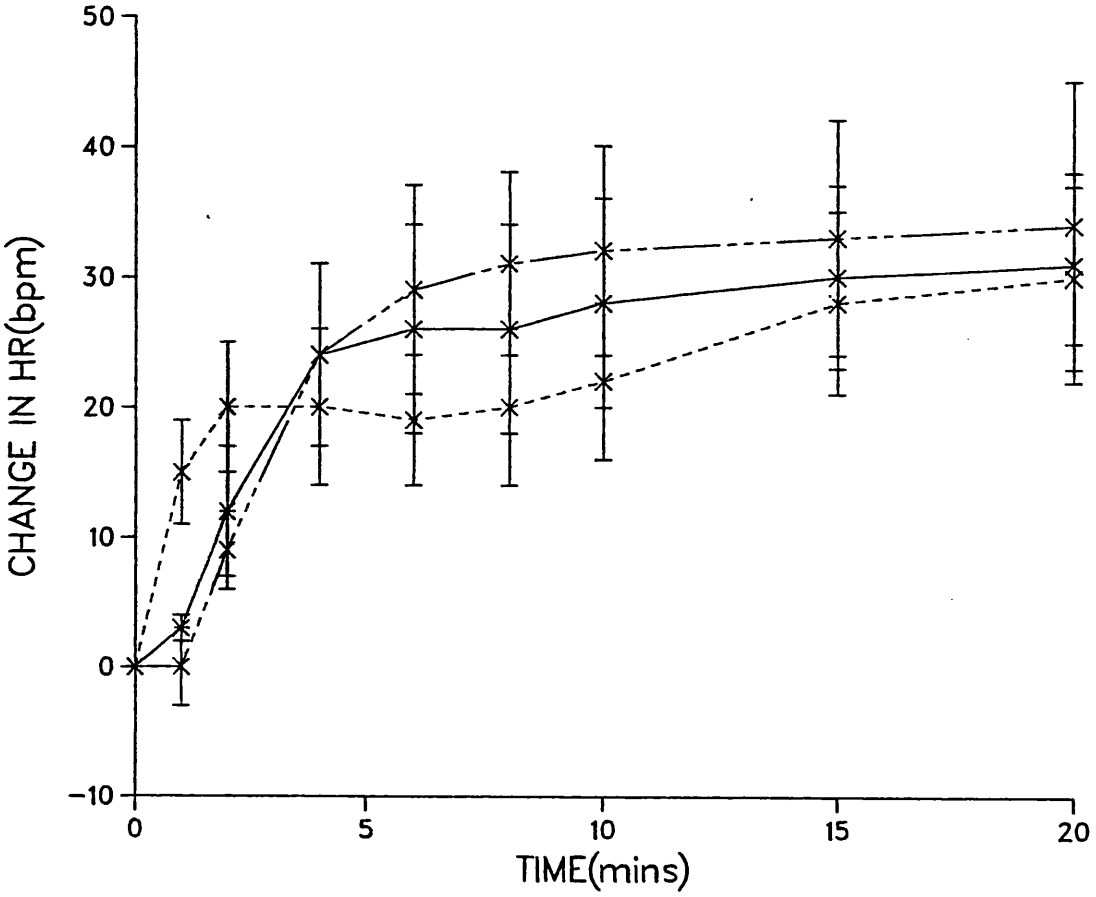

Figure 11b.

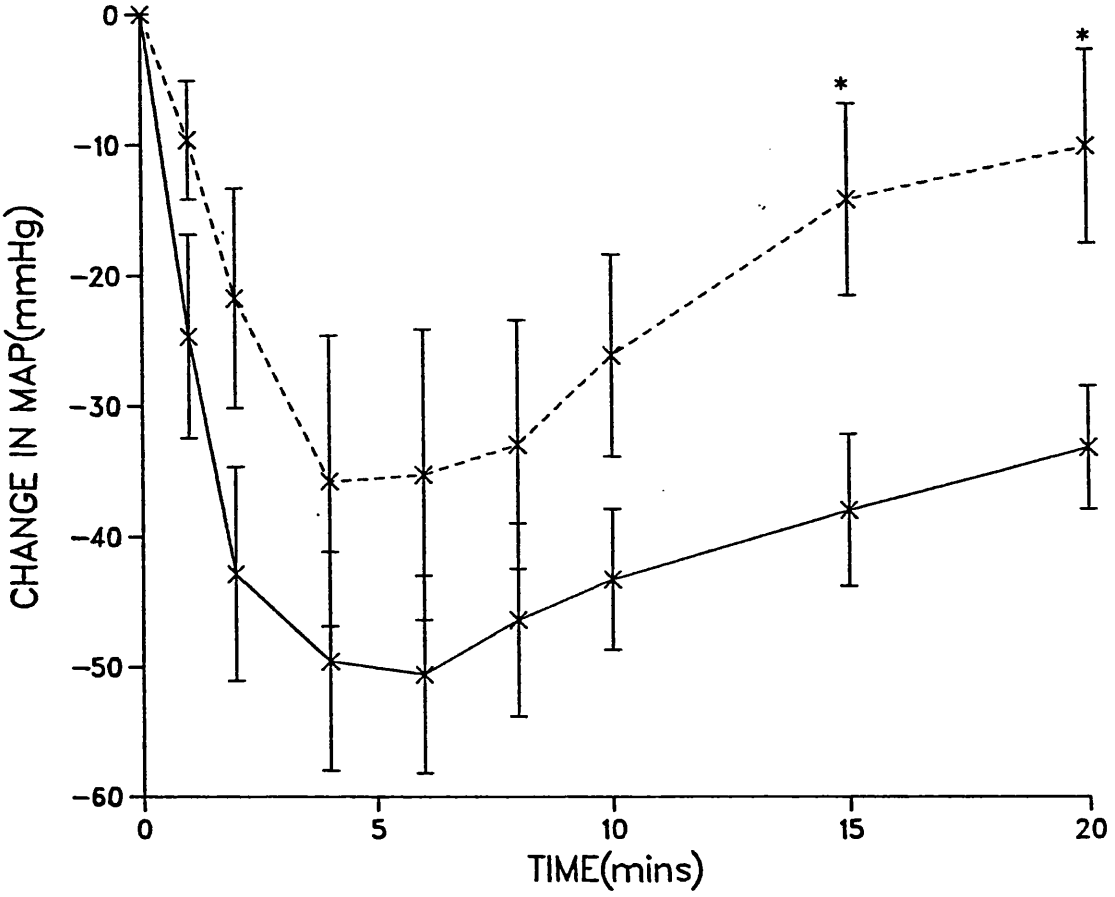

Figure 12a.

Figures 12a and 12b. Change in mean arterial pressure and heart rate following icv injection of 20 mcg isoprenaline in anaesthetised New Zealand rats.

x———x No pretreatment (n=7) 110 mmHg, 369 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 95 mmHg, 389 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$

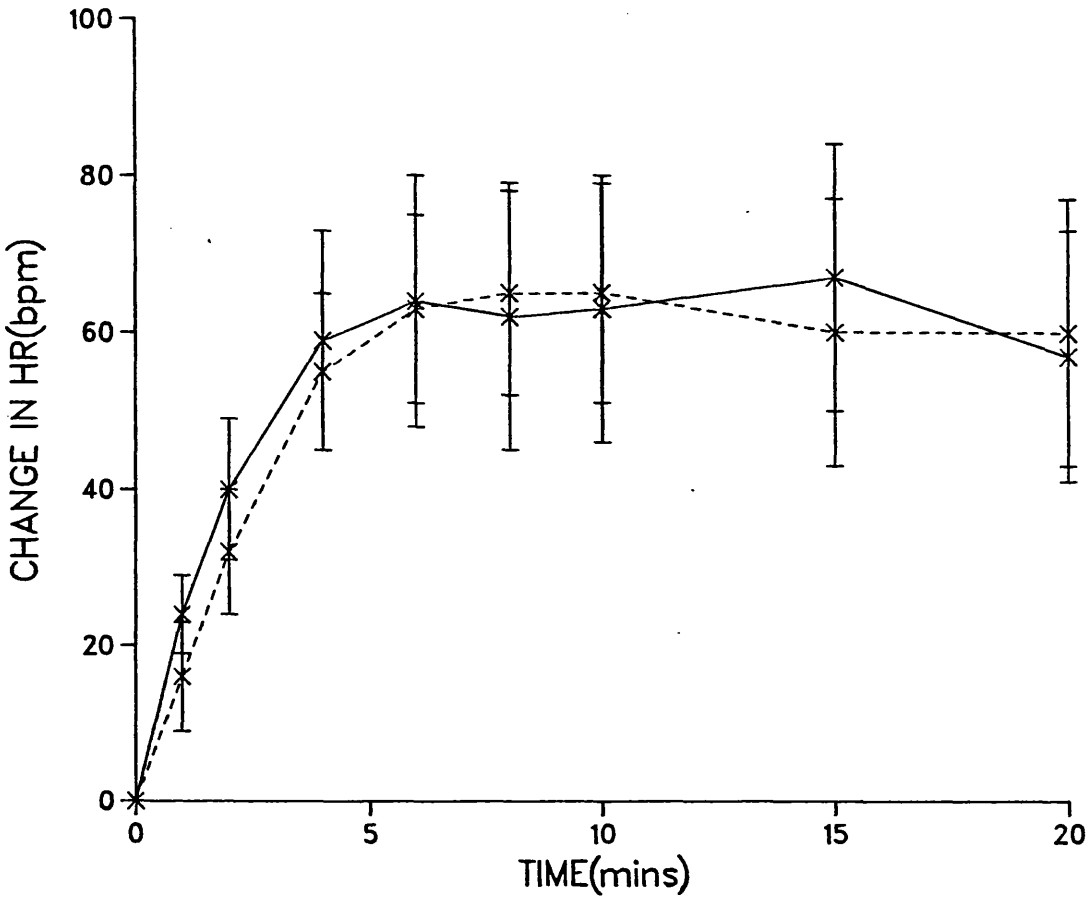

Figure 12b.

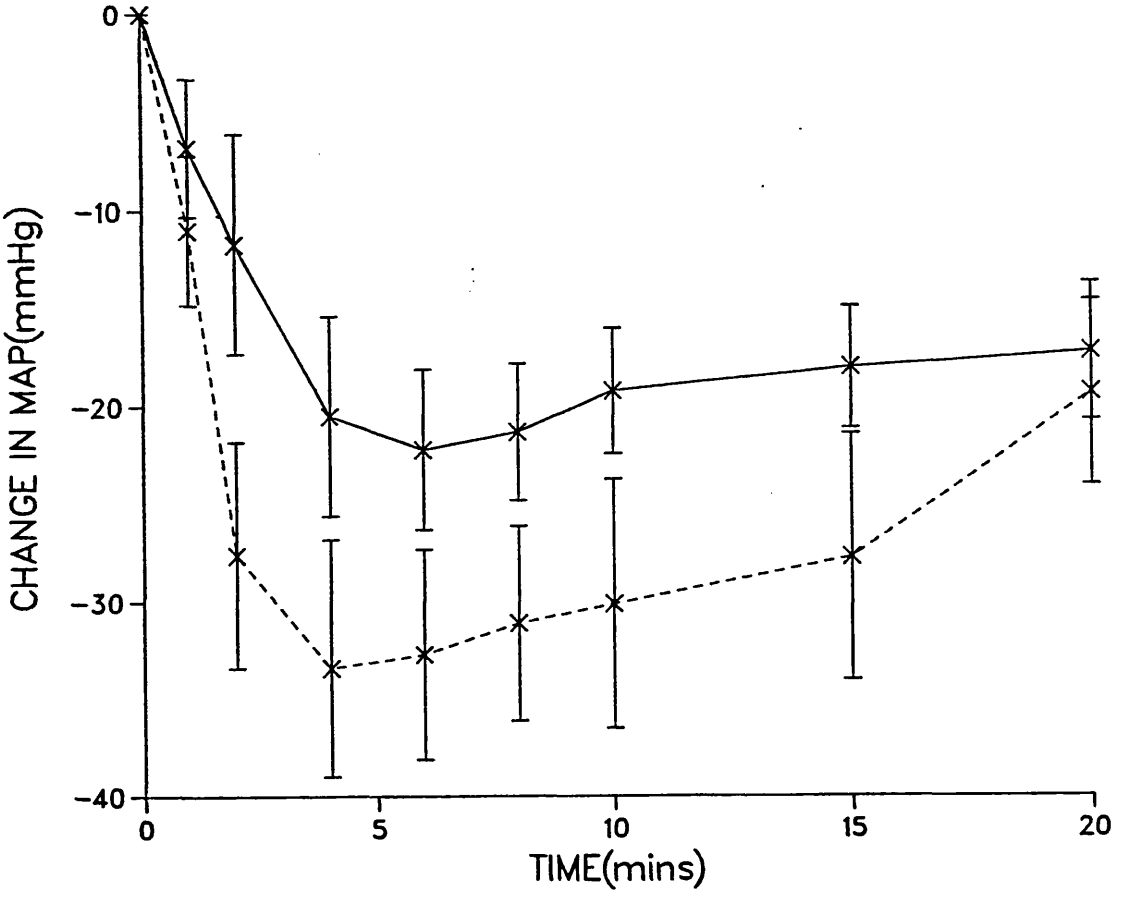

Figure 13a.

Figures 13a and 13b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in anaesthetised New Zealand rats.

x———x Intact animals (n=6) 87 mmHg, 427 bpm.

x- - - - - -x Spinal cord transected at C_2 level and bilateral vagotomy (n=7) 61 mmHg, 376 bpm.

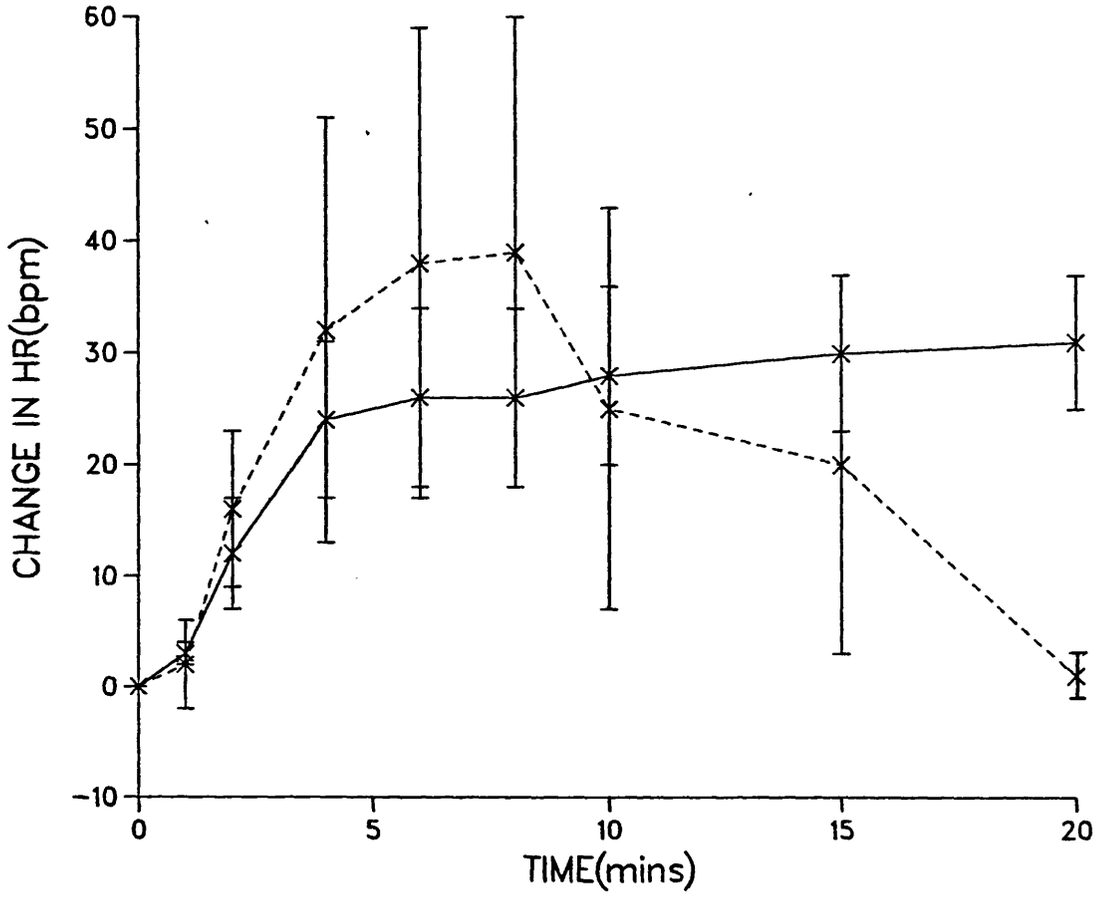

Figure 13b.

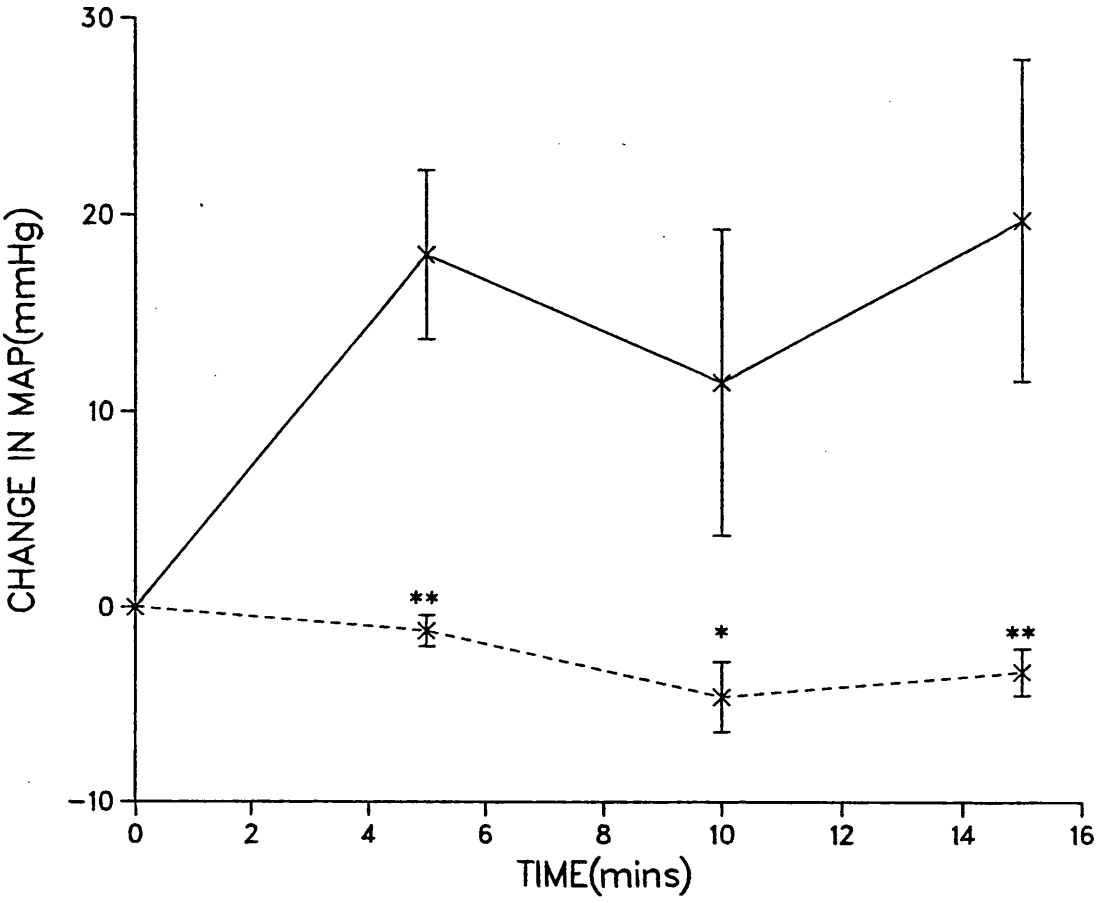

Figure 14a.

Figures 14a and 14b. Change in mean arterial pressure and heart rate following icv injection of 30 mcg propranolol in anaesthetised New Zealand rats.

x————x Intact animals (n=6) 92 mmHg, 441 bpm.

x- - - - - -x Spinal cord transected at C2 level and bilateral vagotomy (n=6) 62 mmHg, 398 bpm.

Significant difference from intact animals denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

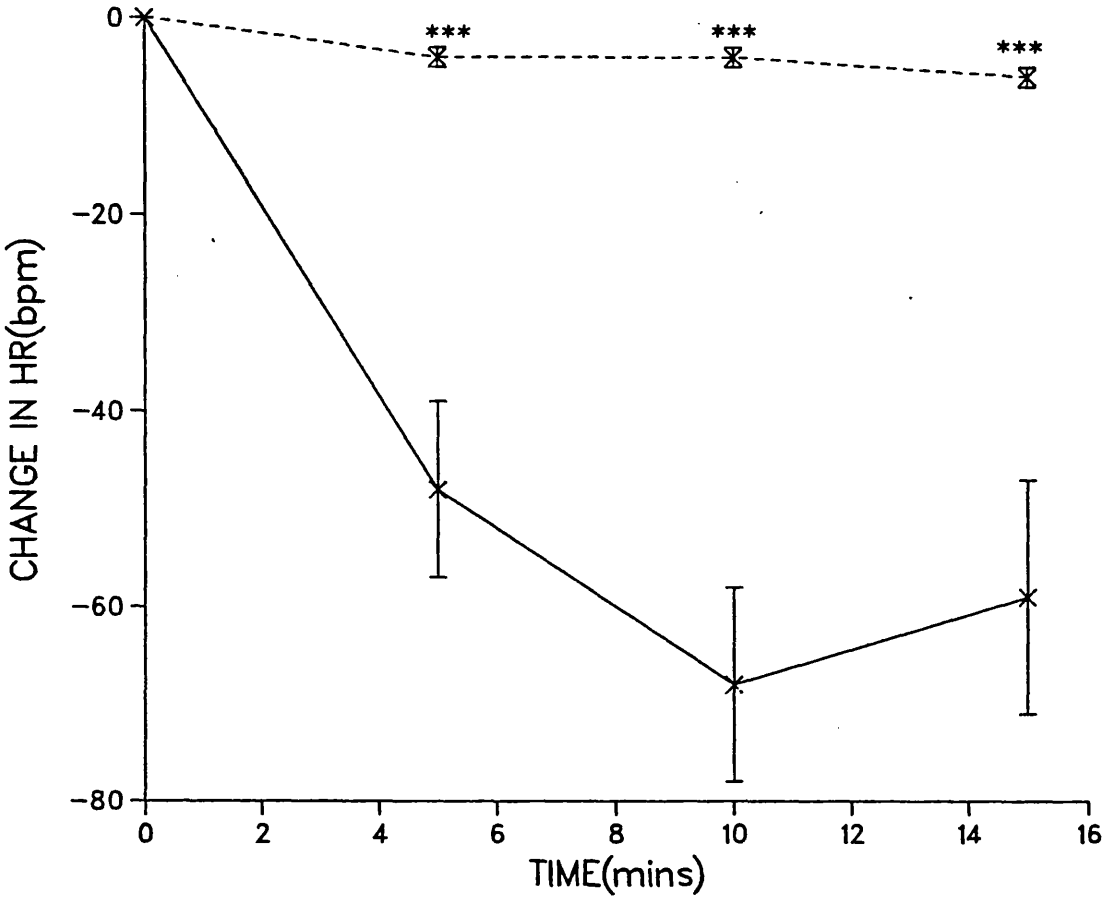

Figure 14b.

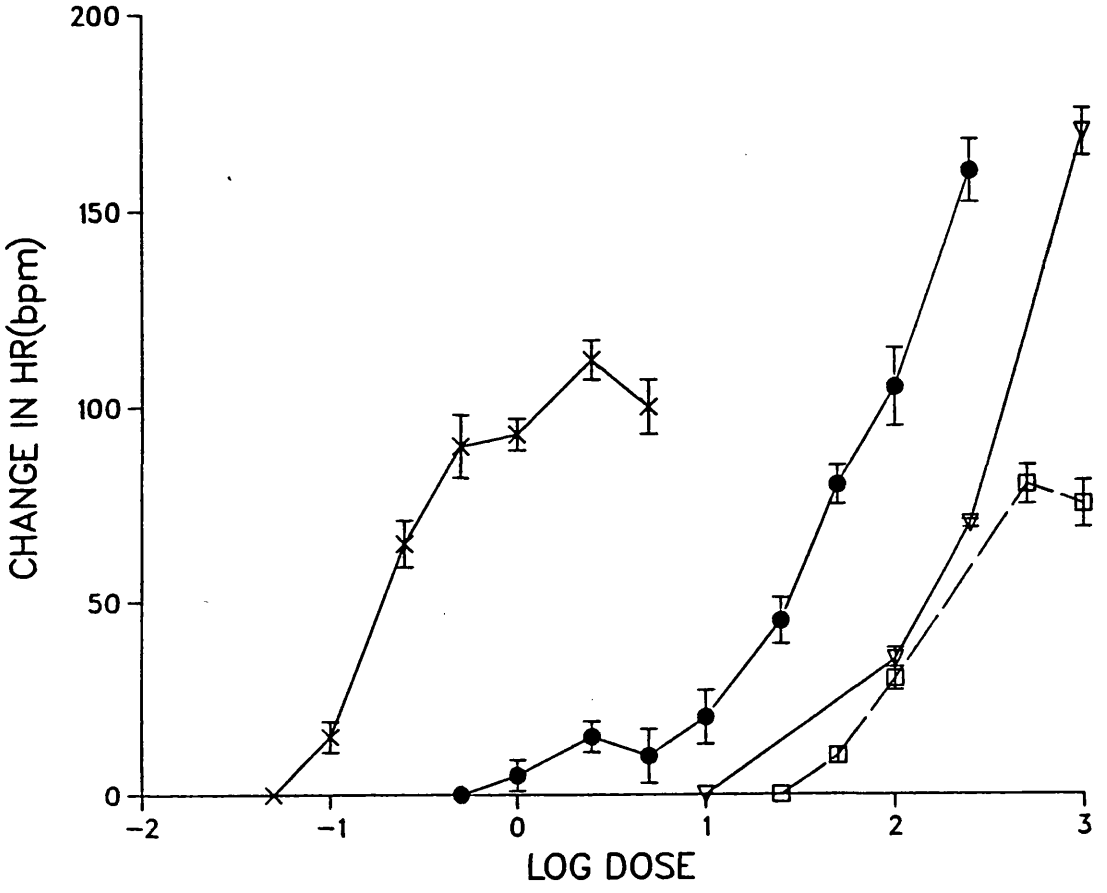

Figure 15. Increase in heart rate against log. dose of isoprenaline (ng) injected intravenously.

x——x No pretreatment (n=4).

●——● 60 mg/Kg propranolol po daily for 14 days, last dose 24 hours before experiment (n=4).

▽——▽ 60 mg/Kg propranolol po daily for 14 days, last dose 1 hour before experiment (n=4).

□— — —□ 60 mg/Kg propranolol po single dose 1 hour before experiment (n=4).

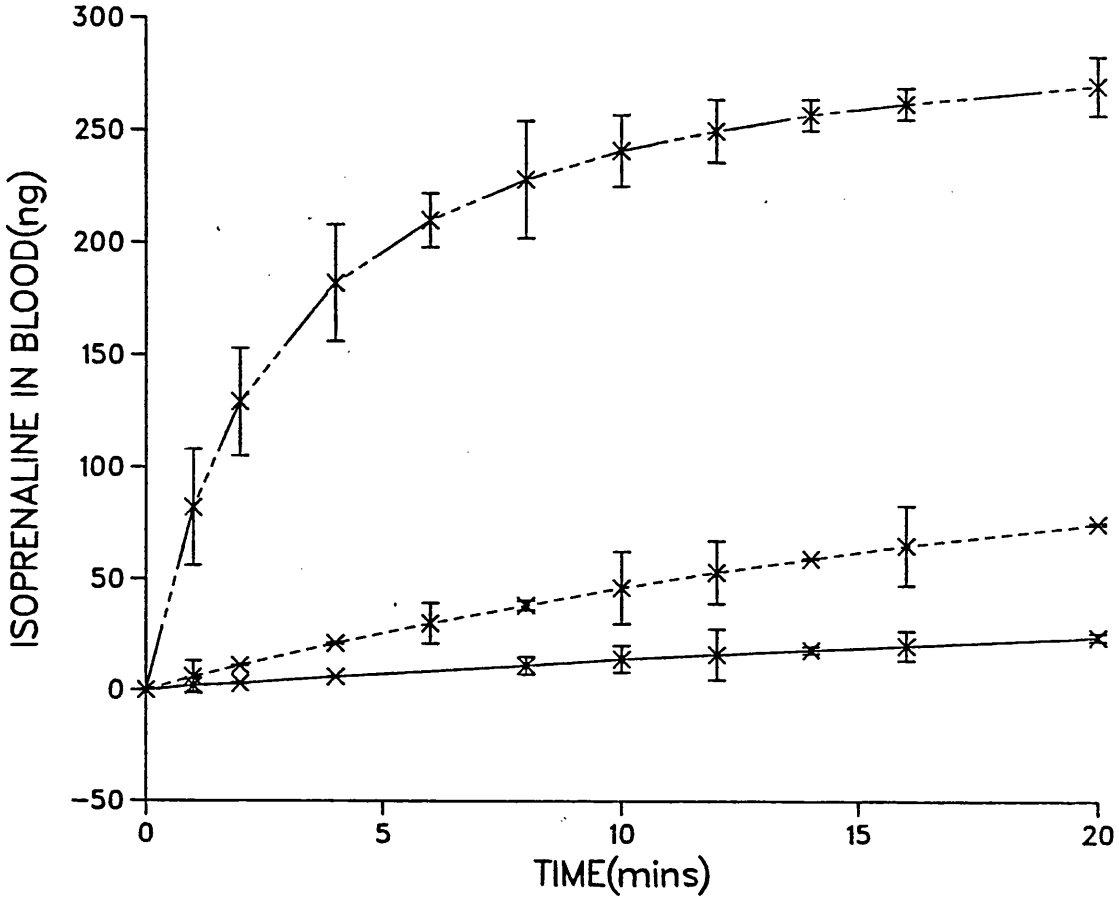

Figure 16. Amount of isoprenaline in the bloodstream of the anaesthetised New Zealand rat following icv injection of radiolabelled drug.

x——————x 1 mcg isoprenaline icv (n=10).

x- - - - - - - - - -x 5 mcg isoprenaline icv (n=10).

x-·-·——·-·-·—x 20 mcg isoprenaline icv (n=10).

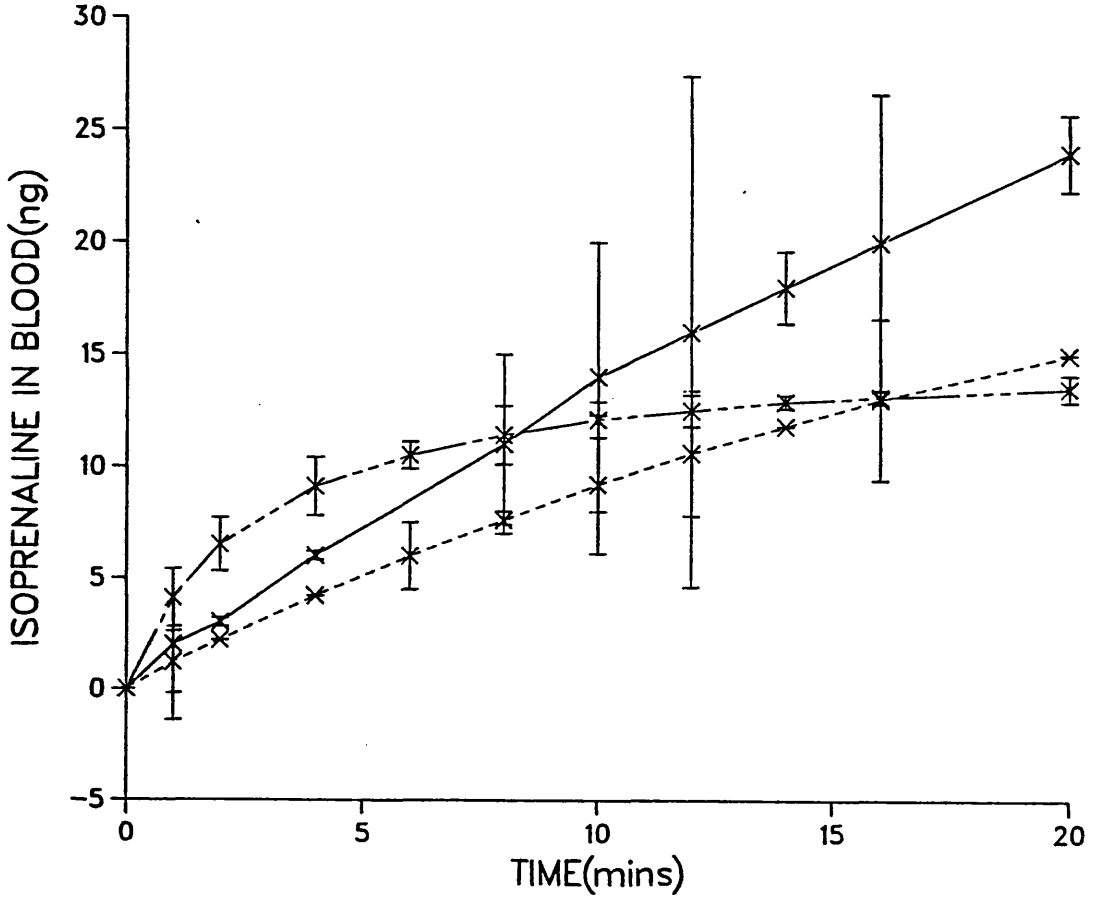

Figure 17. Amount of isoprenaline in the bloodstream per milligram of isoprenaline injected icv in anaesthetised New Zealand rats.

x————————x 1 mcg isoprenaline icv (n=10).

x- - - - - - - - -x 5 mcg isoprenaline icv (n=10).

x—·····—·····—x 20 mcg isoprenaline icv (n=10).

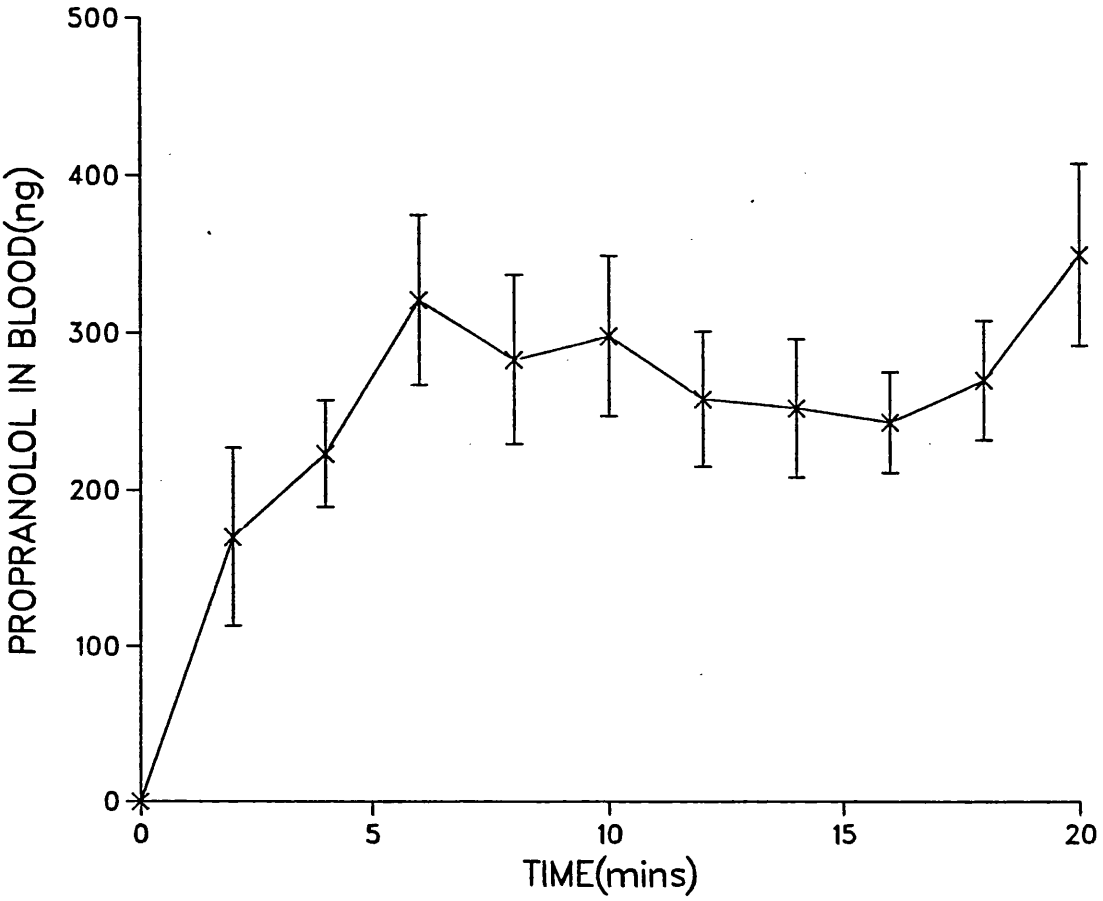

Figure 18. Amount of propranolol in the bloodstream following icv injection of 30 mcg radiolabelled propranolol in anaesthetised New Zealand rats (n=10).

Tissue / Time	Brain	Heart	Lungs	Liver	Kidneys	Mesenteric Bed
5	23.6µg ± 0.04	0.3µg ± 0.03	1.85µg ± 0.20	0.82µg ± 0.09	0.45µg ± 0.02	0.13µg ±0.01
	78.7%	1.0%	6.2%	2.7%	1.5%	0.4%
10	17.04µg ± 0.18	0.27µg ± 0.03	1.86µg ± 0.26	2.09µg ± 0.19	0.69µg ± 0.03	0.12µg ±0.03
	66.8%	0.9%	6.2%	7.0%	2.3%	0.4%
15	20.97µg ± 0.68	0.96µg ± 0.05	1.76µg ± 0.22	2.09µg ± 0.21	1.15µg ± 0.08	0.04µg ±0.01
	70.0%	3.2%	5.9%	7.0%	3.8%	0.1%
20	21.58µg ± 1.02	0.98µg ± 0.12	1.67µg ± 0.32	2.00µg ± 0.08	0.75µg ± 0.03	0.12µg ±0.04
	71.9%	3.3%	5.6%	6.7%	2.5%	0.4%
30	20.49µg ±0.94	0.83µg ±0.02	2.19µg ±0.10	3.36µg ±0.32	1.31µg ±0.03	0.10µg ±0.06
	68.2%	2.8%	7.3%	11.2%	4.4%	0.3%

Figure 19. Amount of propranolol present in tissues following icv injection of 30 mcg ^{14}C propranolol in anaesthetised New Zealand rats, expressed as micrograms (µg) (mean +/- standard error) and percentage of injected dose at times following icv injection.

Tissue / Time	Brain	Heart	Lungs	Liver	Kidneys
5	2.89µg ± 0.21	0.09µg ± 0.01	0.03µg ± 0.01	0.05µg ± 0.02	0.03µg ± 0.03
	57.7%	1.8%	0.6%	1.0%	0.07%
10	4.22µg ± 0.17	0.12µg ± 0.03	0.13µg ± 0.03	0.09µg ± 0.04	0.06µg ± 0.02
	84.3%	2.4%	2.6%	1.9%	1.1%
15	4.08µg ± 0.36	0.12µg ± 0.05	0.17µg ± 0.05	0.14µg ± 0.06	0.06µg ± 0.01
	81.6%	2.4%	3.4%	2.9%	1.2%
20	3.74µg ± 0.76	0.19µg ± 0.04	0.15µg ± 0.06	0.2µg ± 0.02	0.1µg ± 0.01
	74.8%	3.8%	3.0%	4.1%	1.9%

Figure 20. Amount of isoprenaline remaining in tissues following icv injection of 5mcg ^{3}H- isoprenaline in anaesthetised New Zealand rats, expressed as micrograms (µg) (mean +/- standard error) and percentage of injected dose at times following icv injection.

3.2.11. Discussion.

3.2.11.1. Icv injection of beta- adrenoceptor blocking agents.

Icv injection of propranolol (30 mcg) produced an increase in mean arterial pressure and a fall in heart rate (see figures 1a and 1b). However, the variation between animals was very high, and in some experiments a fall in mean arterial pressure was observed, which was thought to be attributable to the accompanying large fall in heart rate in these animals.

This increase in mean arterial pressure following icv injection of propranolol is in conflict with the results of previous studies reported in anaesthetised animals. A fall in arterial pressure has been reported in anaesthetised rats (Cohen et al, 1979) anaesthetised dogs (Privitera et al, 1979; Tackett et al, 1985) and anaesthetised cats (Klevans et al, 1976).

In all these studies, bradycardia was obtained following icv injection of propranolol, as was found in this study.

These effects following icv propranolol appear to be centrally mediated, since following transection of the spinal cord and vagus nerves, the responses were abolished (see figures 14a and 14b).

Icv injection of atenolol had little effect on mean arterial pressure and heart rate, suggesting that central beta1-adrenoceptors may not play an important role in cardiovascular regulation. ICI 118,551 produced a biphasic change in mean arterial pressure, accompanied by tachycardia. Whether the tachycardia was a reflex response to the initial fall in mean arterial pressure is not known. There is no information available from other workers regarding icv injection of these drugs.

3.2.11.2. Iv injection of beta- adrenoceptor blocking agents.

The bradycardia following iv injection of 12 mcg propranolol was significantly greater than that following icv injection of 30 mcg ($p<0.05$). This conflicts with results obtained by Klevans et al (1976). In anaesthetised vagotomised cats, they found that icv injections of 3 and 5 mM dl- propranolol produced significantly greater responses than corresponding iv injections. The increase in mean arterial pressure was found to be comparable following icv or iv injection of propranolol (see figures 2a and 2b).

Following iv injection of 12 mcg atenolol, a marked bradycardia and hypotension were observed. this is a strong contrast to the slight responses observed following icv injection. Atenolol is highly water soluble and unlikely to penetrate into the central nervous system to any great extent

(Barrett, 1977). It is also devoid of any membrane stabilising action. Thus any effect seen following iv injection of atenolol is likely to be from blockade of peripheral beta1- adrenoceptors.

Taylor and co-workers (1981) found very low levels of atenolol in the cerebrospinal fluid of human subjects; it is possible that the rate of penetration of atenolol into cerebrospinal fluid is so low that renewal of cerebrospinal fluid by bulk flow is sufficiently rapid to prevent a 'true' equilibrium being established.

Further evidence for the lack of penetration of atenolol into the central nervous system arises from the fact that adverse reactions such as psychosis, depression, hallucinations and nightmares are rare with atenolol (Simpson, 1977; Henningsen and Mattiasson, 1979), whereas psychotic problems and central nervous system related side effects appear to arise with lipid soluble beta- adrenoceptor blocking agents which enter the brain in high concentrations (Fleminger, 1978; Hinshelwood, 1969; Shaw and England, 1977).

In these experiments, it appears that little atenolol leaks from the brain to the periphery since following icv injection, the marked bradycardia produced by iv injection was absent. However, leakage of atenolol from the brain was not evaluated in this study.

Iv injection of ICI 118,551 produced an increase in mean arterial pressure and a transient fall in heart rate; this contrasts with the tachycardia observed following icv injection of ICI 118,551. There is no information available regarding the extent to which ICI 118,551 will cross the blood brain barrier following peripheral administration, but it has been reported that it has a membrane stabilising action similar to that of propranolol (Bilski et al, 1983).

3.2.11.3. Icv injection of propranolol and adrenaline.

Icv injection of 20 mcg adrenaline produced a transient increase in mean arterial pressure and a significant bradycardia (see figures 3a and 3b). The bradycardia was unaffected by pretreatment with 30 mcg propranolol icv but a significant potentiation of the hypertension was seen. These findings agree with those reported by Clough et al (1981a; 1981b) using thiobutobarbitone anaesthetised Wistar rats. These authors suggested that icv adrenaline exerted an effect on blood pressure and heart rate by interaction with central alpha- and beta- adrenoceptors. Blockade of central beta-adrenoceptors by icv injection of a beta- adrenoceptor blocker would then enable the expression of mainly alpha- adrenoceptor mediated effects, i.e. an increase in blood pressure.

Borkowski and Finch (1977; 1978; 1979) demonstrated a

hypotension and bradycardia in conscious and anaesthetised spontaneously hypertensive rats following icv injection of adrenaline (1-20 mcg). The degree of hypotension and bradycardia was greater in anaesthetised than in conscious rats. In conscious rats the responses were antagonised by pretreatment with propranolol, whereas in anaesthetised rats the responses were not significantly affected. In both groups, pretreatment with alpha- adrenoceptor antagonists did not significantly alter the responses. They concluded that the depressor effects of icv adrenaline were mediated by central adrenoceptors and, in conscious rats, these were mediated by beta- rather than alpha- adrenoceptors.

A difference in the response to icv adrenaline observed between anaesthetised and conscious rats was also highlighted by Correa et al (1982). They found that the hypotension observed following icv injection of adrenaline was greater in anaesthetised than conscious rats. They also demonstrated a reversal of the hypotension produced by 120 nmol adrenaline icv by the pretreatment with 400 nmol propranolol icv in urethane anaesthetised rats.

In conscious rabbits, Toda et al (1969) found a centrally mediated hypotensive action in response to 200 mcg adrenaline icv.

In conclusion, bradycardia has been reported in all studies of

the effects of icv adrenaline regardless of species, strain or presence of anaesthesia. However, there are conflicting results regarding changes in arterial pressure following icv adrenaline, although it can be generalised that pretreatment with propranolol serves to either reduce hypotension or potentiate hypertension, indicating that adrenaline may interact with both central alpha- and beta- adrenoceptors. Pretreatment with a beta- adrenoceptor blocker would thus potentiate alpha- adrenoceptor mediated effects, resulting in enhanced increases in blood pressure.

3.2.11.4. Icv injection of beta- adrenoceptor agonists: Effect of pretreatment with beta- adrenoceptor blocking drugs.

One of the most important considerations in studies using icv injection of drugs is how much will leak into the peripheral circulation following injection. If this variable is unknown, it is difficult to discriminate between centrally and peripherally mediated actions of a drug following icv injection. This can be evaluated in two ways:

1. Spinal transection and bilateral vagotomy. It is reasoned that, if any effect on blood pressure and heart rate is abolished by this operation, then the responses to an icv injection must be centrally mediated. This reasoning has one important failing; it only holds true if the responses are mediated via the sympathetic or parasympathetic nervous systems. It is possible that a central action of an icv injected drug may result in a release of a humoral transmitter into the peripheral blood stream which may then interact with peripheral receptors to cause changes in cardiovascular parameters. Transection of the spinal cord and vagus nerves would not abolish this centrally mediated effect.

2. Direct evaluation of the amount of drug leaking from the brain into the peripheral circulation following icv injection. This can be achieved by injection of radiolabelled drugs into the cerebral ventricle and subsequent evaluation of the amount of radioactivity in the brain. If the amount of drug leaking into the periphery is known, studies can be carried out with peripheral injections of equivalent amounts to determine any difference in responses following central and peripheral injections of the drugs in question.

In this study, the amount of propranolol and isoprenaline leaking from the brain to the periphery was measured following the icv injection of radiolabelled isotopes.

The levels of radioactivity present in the brain indicated that, following icv injection into anaesthetised New Zealand rats, there was at least 60% of the injected dose remaining in the brain (see figures 19 and 20). It was decided that in future studies 40% of the icv dose of a beta- adrenoceptor blocking drug could be given intravenously to give an equivalent peripheral effect.

It was found that, for both propranolol and isoprenaline, similar concentrations of drug were present in both the blood stream and various body organs. The amount in the blood stream was found to be approximately 1%, regardless

of the dose or drug injected (see figures 17 and 18).

The passage of drugs from cerebrospinal fluid to the blood stream is only partly dependant on lipid solubility, and compounds with a low lipid solubility leave the cerebrospinal fluid almost as rapidly as those with a high lipid solubility (Mayer et al, 1960). Leakage of drugs from the cerebrospinal fluid into the systemic circulation is by the process of bulk flow across the extremely porous arachnoid villi from the sub-arachnoid space into the dural venous sinuses (Schanker, 1962). The observation that isoprenaline and propranolol appear to leave the brain to similar degrees agrees with this principle.

Icv injection of isoprenaline (1-20 mcg) produced a dose dependant hypotension and accompanying tachycardia in anaesthetised New Zealand rats (see figures 7,8 and 12). These results agree with other studies using anaesthetised animals. Hypotension and tachycardia following icv isoprenaline have been reported in anaesthetised dogs (Bhargava et al, 1972), cats (Gagnon and Melville, 1966; 1967), rabbits (Toda et al, 1969) and rats (Cohen et al, 1979; Peres-Polon and Correa, 1984).

Pretreatment with 30 mcg propranolol icv did not significantly attenuate the hypotension produced by 20 mcg isoprenaline icv (see figure 12a), whereas that produced

following icv injection of 1 and 5 mcg isoprenaline was only significantly reduced at a few time points (see figures 7a and 8a). Pretreatment with 12 mcg propranolol iv had no effect on the hypotension produced by 5 mcg isoprenaline icv (see figure 8a). Increasing the dose of propranolol to 60 mcg icv significantly reversed the hypotension produced by 1 mcg isoprenaline icv. An equivalent iv dose of 24 mcg propranolol attenuated the hypotension to approximately the same degree as that following pretreatment with 30 mcg propranolol icv.

Icv injection of propranolol attenuated the hypotension produced by icv isoprenaline to a greater extent than an equivalent iv dose. However, until a very high dose of propranolol is injected icv, this response is resistant to propranolol. This has previously been reported by Nomura (1976) and Peres-Polon and Correa (1984). It is possible that there exists a propranolol insensitive central component involved in the depressor response to isoprenaline, in addition to that caused by interaction with central beta- adrenoceptors. This mechanism could involve a non-neuronal mechanism in which a humoral substance is released into the peripheral bloodstream. This is supported by the fact that transection of the spinal cord and vagus nerves did not abolish the responses to icv isoprenaline, although the possibility that enough isoprenaline is present in the bloodstream to exert a

peripheral response cannot be ruled out entirely (See figures 13a and 13b). This propranolol insensitive mechanism does not appear to be present in other species since the hypotensive response to isoprenaline was blocked by icv propranolol in the cat (Gagnon and Melville, 1967) and in the dog (Bhargava et al, 1972).

The tachycardia produced by icv isoprenaline was largely unaffected by pretreatment with icv propranolol, but can be potentiated in some cases (see figure 8b). This potentiation may be a result of the large bradycardia caused by pretreatment with propranolol creating an enhanced capacity for increase in heart rate in response to icv isoprenaline.

The responses to 5 mcg isoprenaline icv were not significantly changed by pretreatment with atenolol, either 30 mcg icv or 12 mcg iv (see figures 10a and 10b). Pretreatment with 30 mcg ICI 118,551 significantly reduced the duration of hypotension, whereas 12 mcg iv had little effect. The tachycardia to isoprenaline remained unchanged (see figures 11a and 11b).

ICI 118,551 is a beta$_2$- adrenoceptor selective antagonist, with an in vitro beta$_2$/beta$_1$- selectivity ratio of 123 (Bilski et al, 1983). Thus, it appears that the hypotensive response to icv isoprenaline is mediated via

beta2- adrenoceptors. This finding is in agreement with the results of Clough et al (1981b) that the central inhibitory effect of adrenaline on cardiovascular responses may be mediated by beta2- adrenoceptors.

Following icv injection of 5 mcg clenbuterol, hypotension and tachycardia were observed which were unaffected by pretreatment with 12 mcg propranolol iv (see figures 4a and 4b). Pretreatment with 30 mcg propranolol icv significantly blocked the hypotension but had no effect on the tachycardia.

Clenbuterol is a partial beta- adrenoceptor agonist with higher affinity for and activity at beta2- adrenoceptors than at beta1- adrenoceptors (Waldbeck and Widmark, 1985). It also exerts local anaesthetic activity in high concentrations (Engelhardt, 1976). Clenbuterol has been found to be highly lipophilic and is therefore very likely to pass into the brain; it is very active in reversing reserpine induced hypothermia in mice, an effect thought to be due to stimulation of central beta- adrenoceptors (Ross, 1980). Following chronic treatment with clenbuterol, a significant decrease in the beta- adrenoceptor density of the cerebral cortex of the rat was reported (Hall et al, 1980; Ordway et al, 1987); suggesting that clenbuterol can pass into the brain and stimulate central beta-adrenoceptors.

The hypotension produced by icv clenbuterol was much more amenable to attenuation by icv propranolol than that following injection of isoprenaline into the cerebral ventricle, further supporting the hypothesis that an additional mechanism is involved in the isoprenaline induced depressor response.

Xamoterol (ICI 118,587) possesses an approximate 100-fold selective affinity for beta$_1$- adrenoceptors (Mian et al, 1985) and increases heart rate by about 43% of the maximum increase produced by isoprenaline (Nuttall and Snow, 1982). It has also been shown to be a competitive antagonist of the chronotropic and vasodilator effects of isoprenaline on the heart and blood vessels.

Icv injection of 5 mcg xamoterol caused a fall in blood pressure and increase in heart rate. The hypotension was significantly reversed by both 30 mcg propranolol icv and 12 mcg propranolol iv, whereas the tachycardia was significantly potentiated by 12 mcg propranolol iv and unchanged by 30 mcg propranolol icv (see figures 5a and 5b). Bilateral vagotomy and pretreatment with 6-hydroxydopamine did not significantly alter the hypotension caused by xamoterol alone or the hypertension caused by xamoterol following icv injection of propranolol. However, the tachycardia produced by xamoterol was

significantly blocked by bilateral vagotomy and 6-hydroxydopamine pretreatment (see figures 6a and 6b). It would appear that the cardiovascular responses observed following icv injection of xamoterol were mediated by peripheral effects of drug leaking from the central nervous system to the periphery, since the hypotension was reversed by both icv and iv administration of propranolol and not significantly altered by bilateral vagotomy and treatment with 6-hydroxydopamine. Unfortunately, there was no radioactive xamoterol available to quantify the amount leaking into the periphery following icv injection.

Chronic oral dosing with propranolol significantly reversed the depressor response to icv isoprenaline although a single oral dose of propranolol did not affect this response (see figure 9a). Dose response curves constructed for the tachycardia produced by iv isoprenaline indicated that a single oral dose of propranolol would block peripheral beta- adrenoceptors, although not to the same extent as with chronic oral dosing. This suggests that the depressor response to icv isoprenaline is centrally mediated, since it is unaffected by the single oral dose of isoprenaline despite the blockade of peripheral beta-adrenoceptors.

Chronic oral dosing with propranolol will block the hypotension produced by icv isoprenaline in contrast to the

resistance of this response to acute dosing or injection of propranolol, thus suggesting a central effect of propranolol after long term dosing which is not seen in the short term. However, at present, there is no evidence as to the possible nature of this mechanism.

In conclusion, icv injection of isoprenaline produced hypotension and tachycardia which appeared to involve both an interaction with central beta2- adrenoceptors and possibly a non-neuronal mechanism which may involve the release of a humoral transmitter into the bloodstream.

One of the considerations in this section has been whether the presence of anaesthesia alters the responses observed. In order to eliminate this possibility, the next section will describe icv injections in conscious New Zealand rats in an attempt to discover if the presence of anaesthesia is masking or eliminating responses to icv injected drugs.

3.3. Icv injection of beta- adrenoceptor agonists and pretreatment with beta- adrenoceptor blocking agents in the conscious New Zealand rat.

3.3.1. Icv injection of isoprenaline and pretreatment with propranolol. (2.2.2. and 2.2.3.)

Injection of isoprenaline into the cerebral ventricle of the conscious New Zealand rat caused a dose dependant hypotension and tachycardia. The maximum values are shown below.

Dose isoprenaline (mcg)	Max. hypotension (mmHg)	Max. tachycardia (bpm)	Figure
0.05	6	55	21a & b
1.0	29	77	22a & b
5.0	31	165	23a & b

Animals injected with artificial csf exhibited a small variation in blood pressure and heart rate since they are allowed to roam freely in the experimental cage.

The hypotension produced by 0.05 mcg isoprenaline was not

significantly different from control animals (see figure 21a), but the tachycardia was significantly different over the first 8 minutes after start of icv injection (see figure 21b).

The hypotension and tachycardia produced by 5 mcg isoprenaline icv was not significantly altered by pretreatment with 30 mcg propranolol icv (see figures 23a and 23b). Over the 20 minutes of the experiment, the responses did not start to return to the baseline, but remained at approximately the level of the maximum response which had been attained after 6 minutes.

Pretreatment with 30 mcg propranolol icv significantly reduced the hypotension ($p<0.001$) and tachycardia ($p<0.05$) produced by 1 mcg isoprenaline icv. 30 mcg propranolol iv did not affect the hypotension, but significantly ($p<0.001$) potentiated the tachycardia over the first 15 minutes of the experiment (see figures 22a and 22b).

3.3.2. Icv injection of propranolol and clenbuterol. (2.2.2.)

Icv injection of 1 mcg clenbuterol produced a fall in mean arterial pressure (13 mmHg) and an increase in heart rate (92 bpm) which attained a maximum 4 minutes following start of icv injection (see figures 24a and 24b). Pretreatment

with 30 mcg propranolol icv significantly reduced the hypotension and potentiated the tachycardia ($p<0.05$) over the first 10 minutes of experiment.

3.3.3. Leakage of drugs from the central nervous system following icv injection. (2.5.)

Following icv injection of radiolabelled propranolol, subsequent analysis of brain samples indicated that propranolol leaked rapidly from the brain, only 22% being left 15 minutes following injection. Detectable levels were measured in heart and lungs. The levels in the liver and kidneys were found to increase with increasing time (see figure 25).

The amount of isoprenaline left in the brain 15 minutes following icv injection was found to be the same as that of propranolol, however, isoprenaline seemed to leak from the brain at a faster pace than propranolol. Very low levels were detected in the heart and a measurable amount detected in the lungs. As with propranolol, the levels detected in the liver and kidneys increased with increasing time (see figure 26).

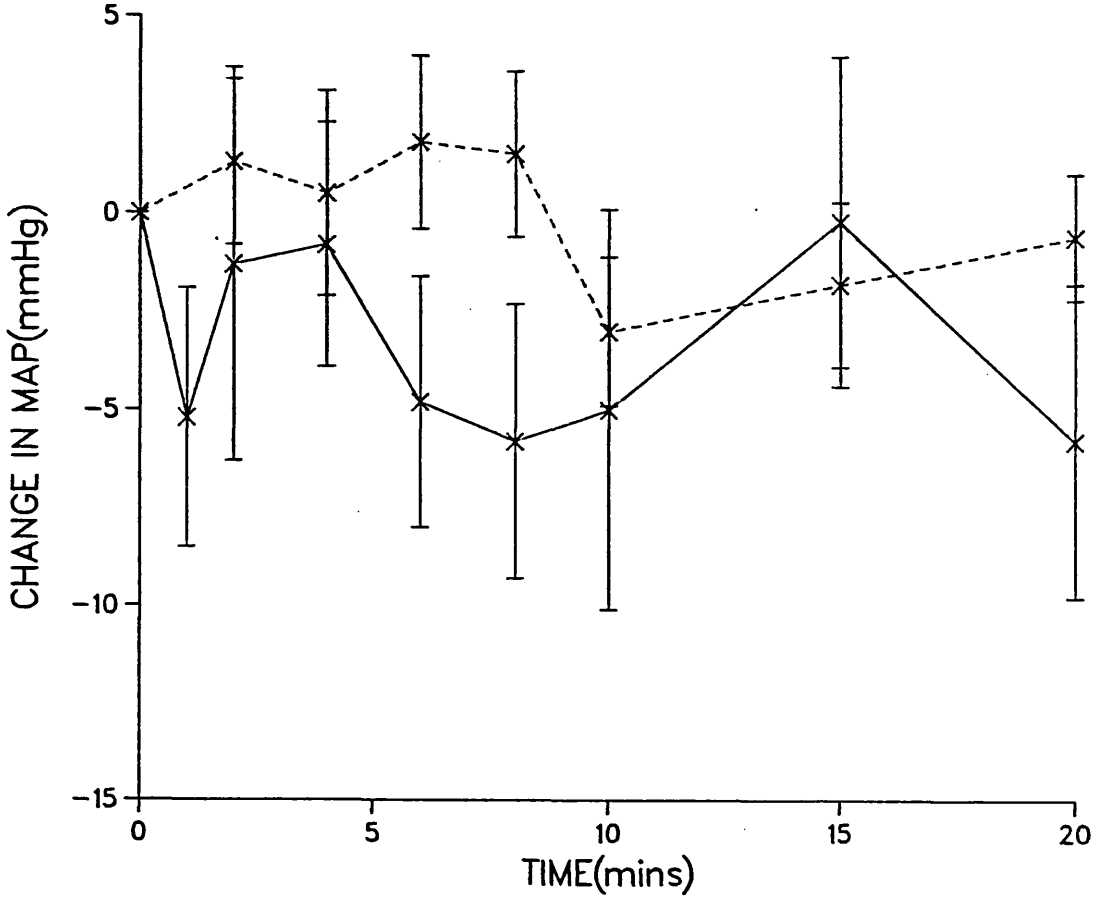

Figure 21a.

Figures 21a and 21b. Change in mean arterial pressure and heart rate following icv injection of 0.05 mcg isoprenaline in conscious New Zealand rats.

x——x 0.05 mcg isoprenaline icv (n=6) 117 mmHg, 388 bpm.

x- - - -x Animals injected icv with vehicle only (n=6) 133 mmHg, 404 bpm.

Significant difference from control animals denoted:

** $p<0.01$ *** $p<0.001$

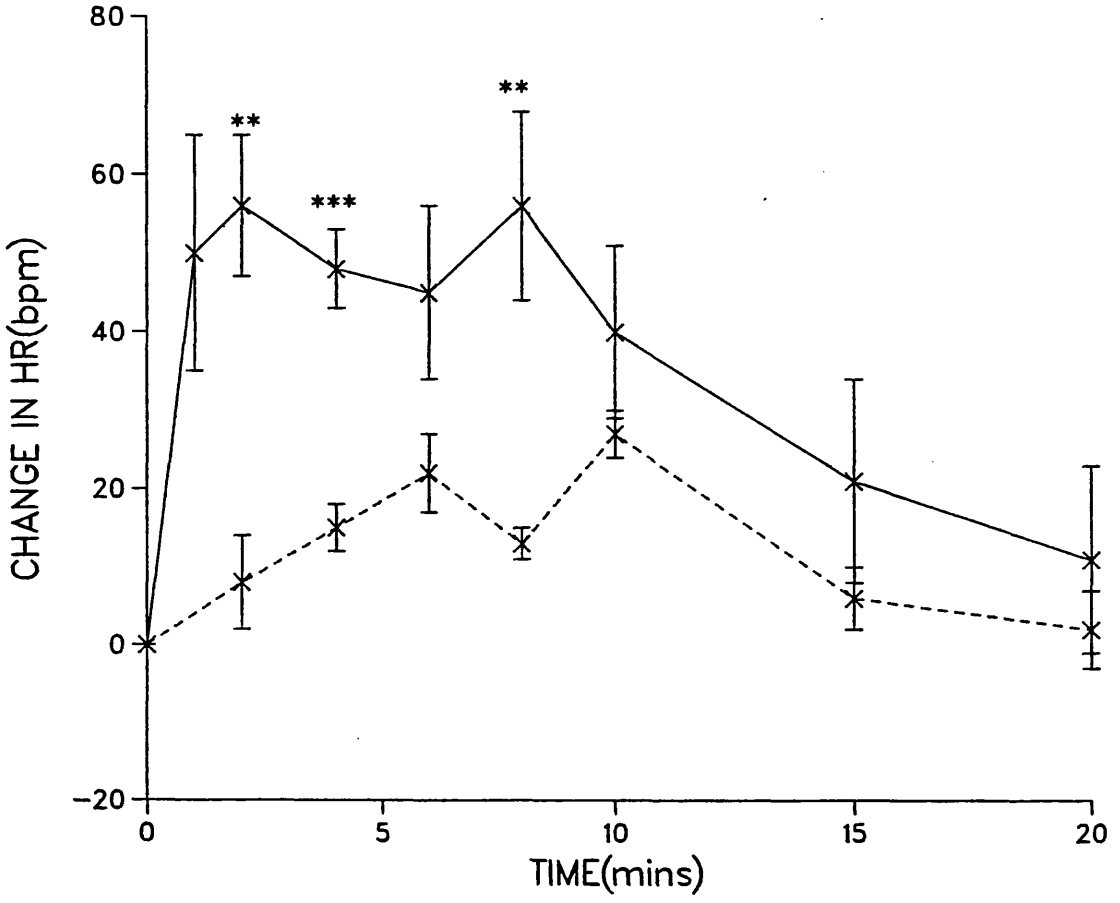

Figure 21b.

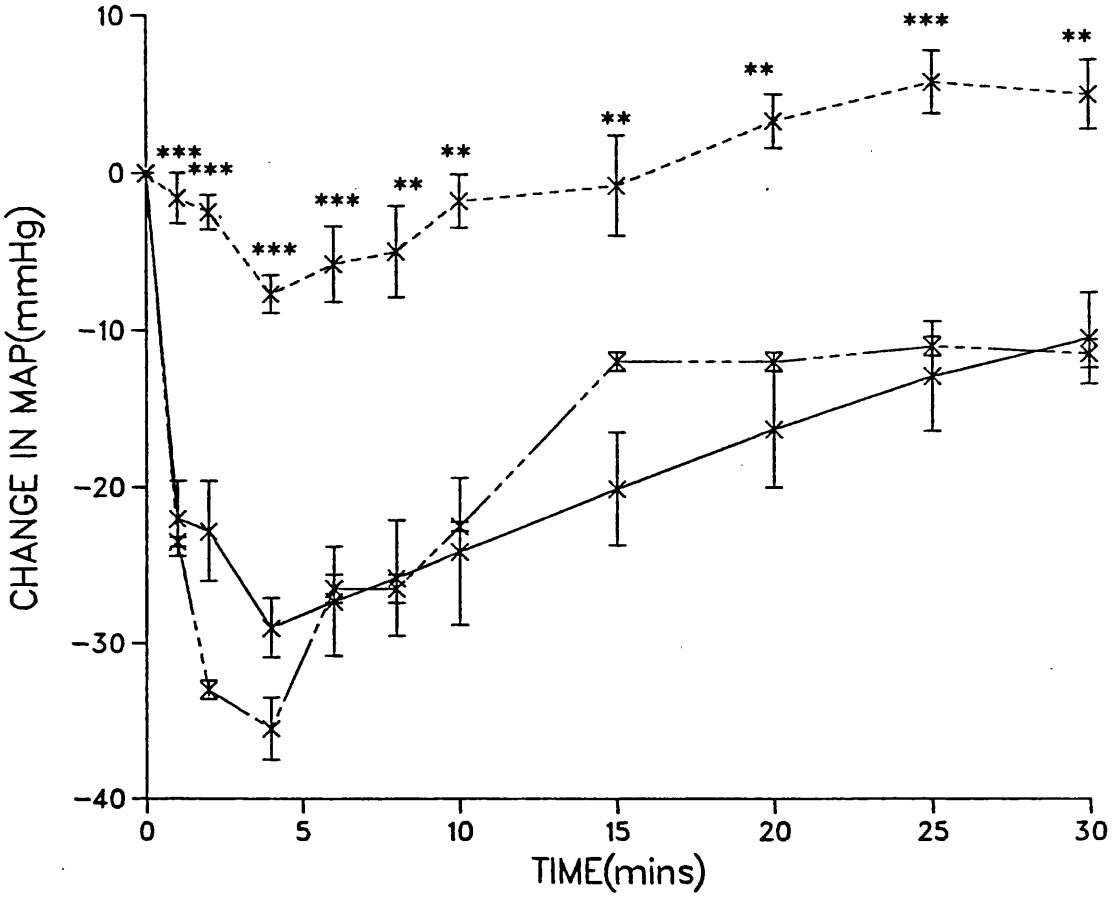

Figure 22a.

Figures 22a and 22b. Change in mean arterial pressure and heart rate following icv injection of 1 mcg isoprenaline in conscious New Zealand rats.

x———x No pretreatment (n=6) 158 mmHg, 427 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 143 mmHg, 350 bpm.

x—·-·—x 30 mcg propranolol iv (n=4) 139 mmHg, 340 bpm.

Significant difference from control animals denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

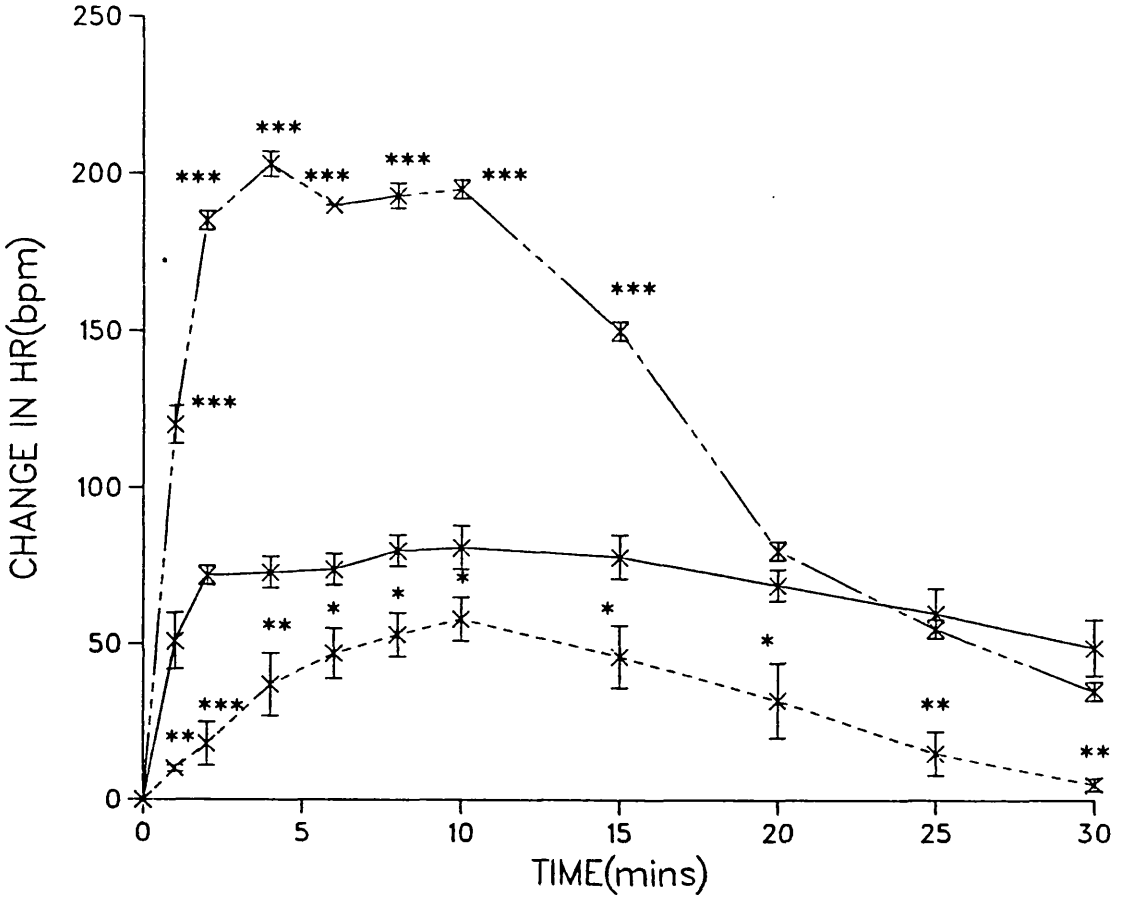

Figure 22b.

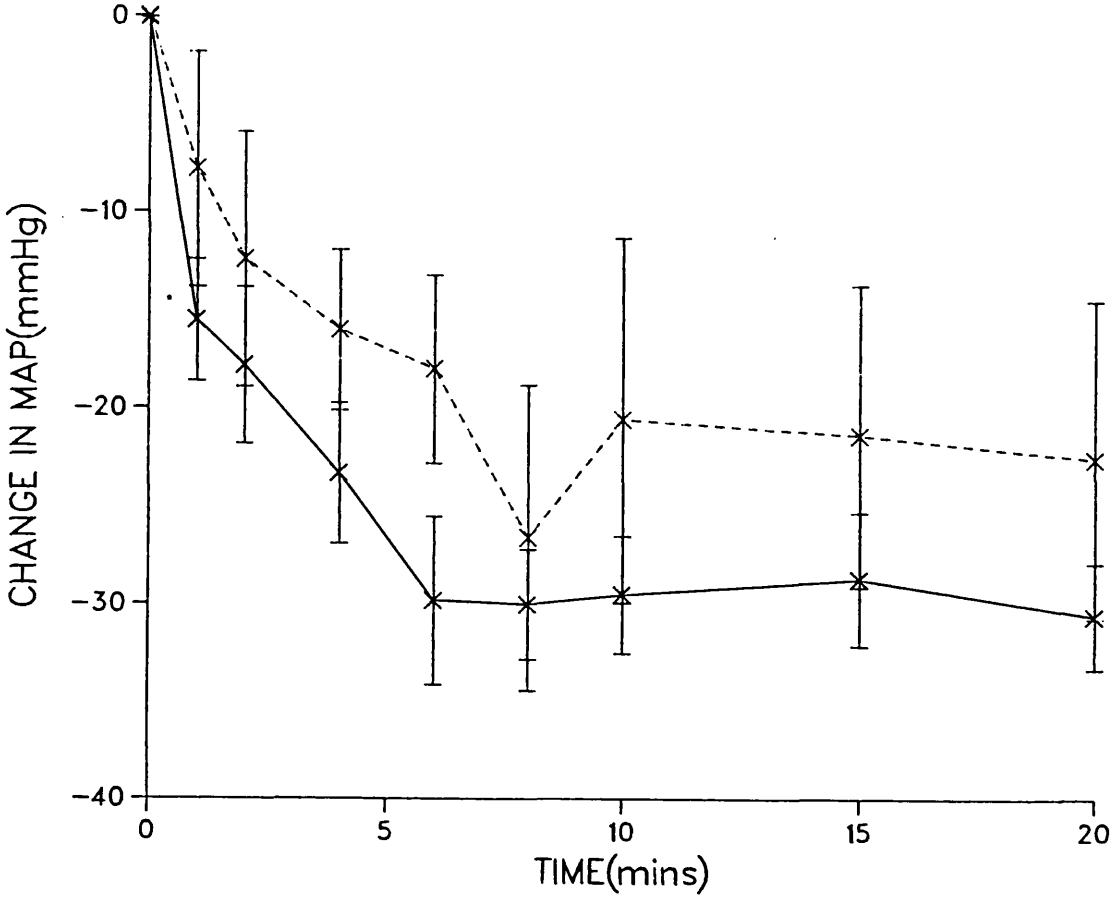

Figure 23a.

Figures 23a and 23b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in conscious New Zealand rats.

x———x No pretreatment (n=6) 113 mmHg, 405 bpm.

x- - - - - -x 30 mcg propranolol icv (n=5) 120 mmHg, 347 bpm.

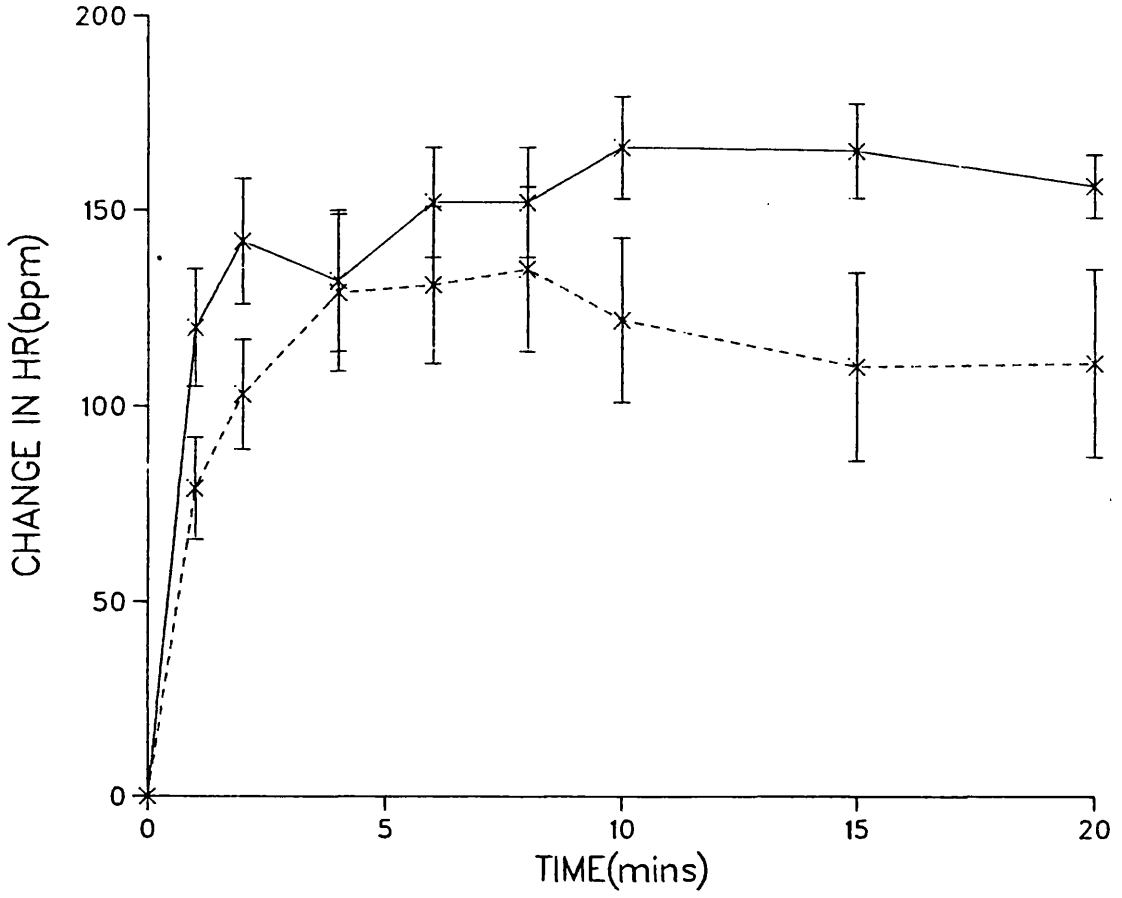

Figure 23b.

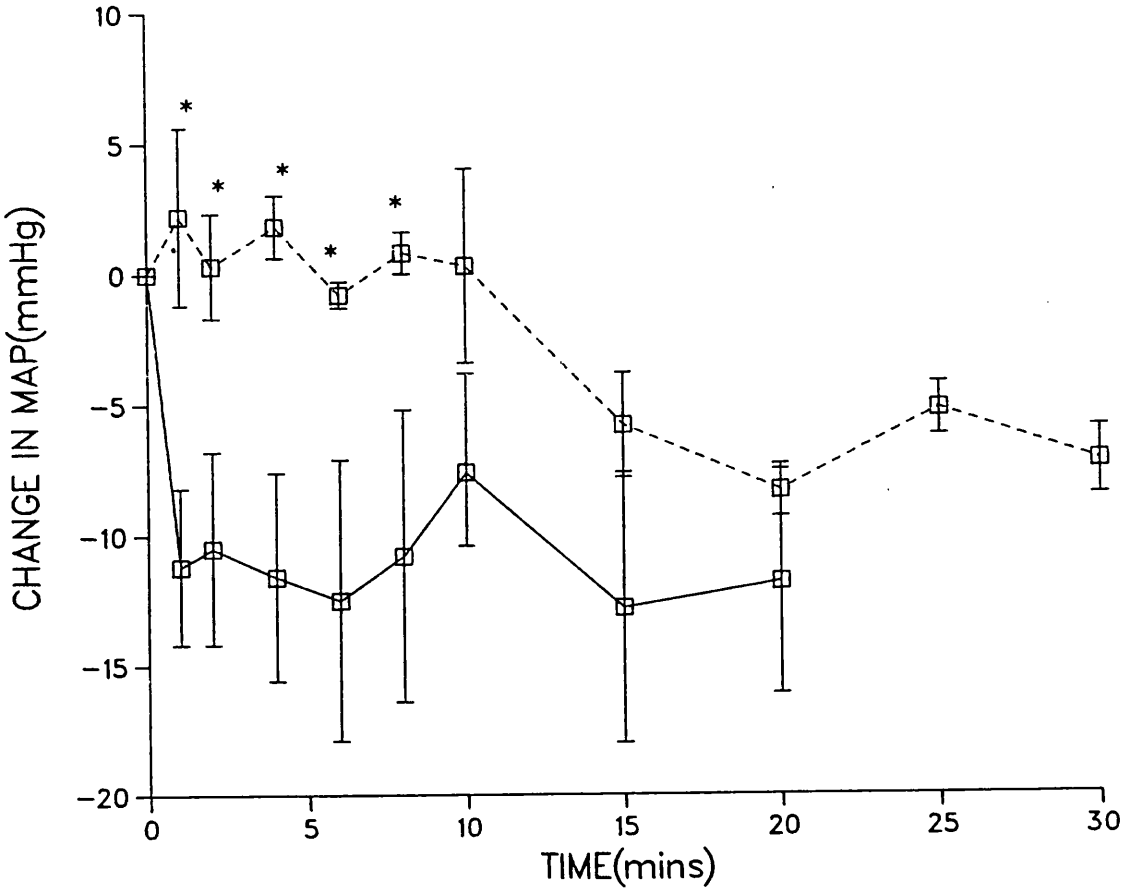

Figure 24a.

Figures 24a and 24b. Change in mean arterial pressure and heart rate produced by 1 mcg clenbuterol icv in conscious New Zealand rats.

⊡——⊡ No pretreatment (n=6) 110 mmHg, 437 bpm.

⊡- - - - - -⊡ 30 mcg propranolol icv (n=7) 122 mmHg, 357 bpm.

Significance from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

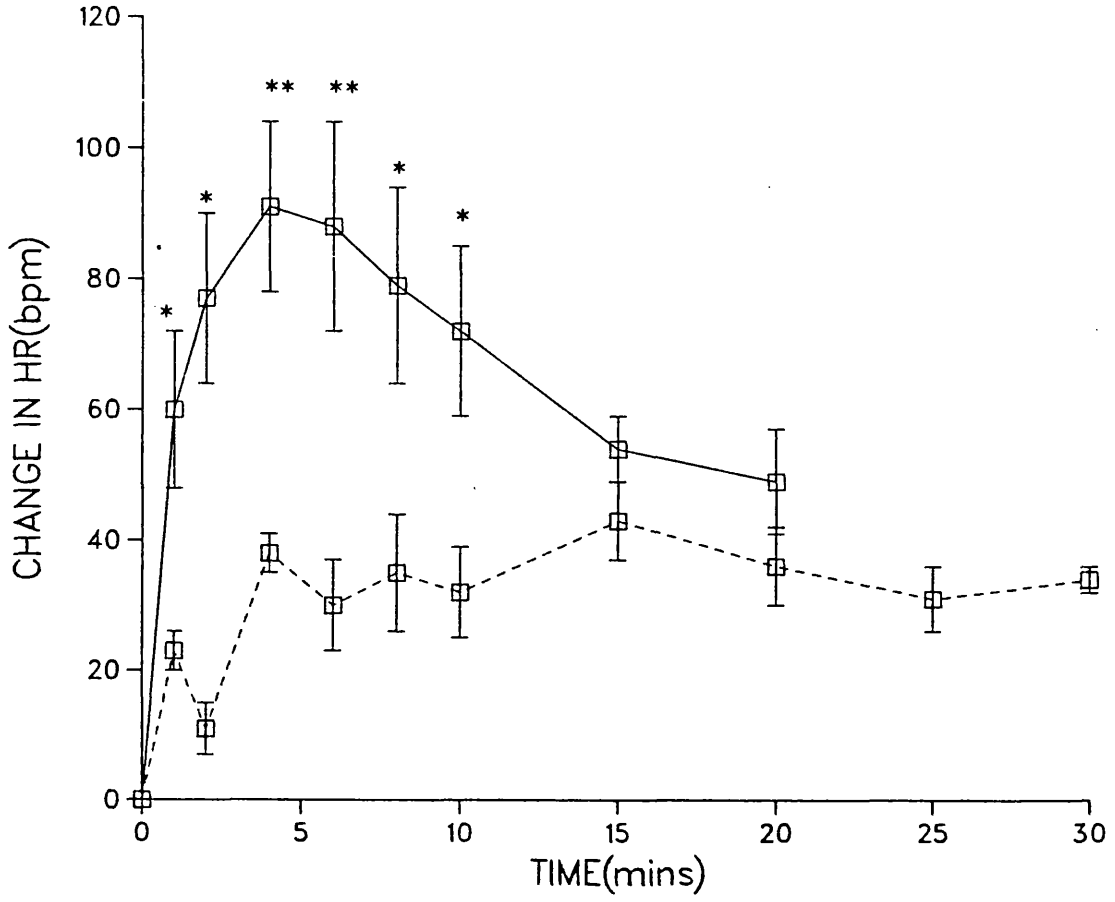

Figure 24b.

Tissue / Time	Brain	Heart	Lungs	Liver	Kidneys	Blood
1	12.47µg ± 0.82	0.17µg ± 0.01	0.74µg ± 0.01	0.40µg ± 0.01	0.33µg ± 0.01	0.84µg ±0.15
	41.5%	0.6%	2.5%	1.3%	1.1%	2.8%
2	13.31µg ± 0.46	0.16µg ± 0.01	0.57µg ± 0.02	1.15µg ± 0.08	0.44µg ± 0.02	0.29µg ±0.02
	44.3%	0.5%	1.9%	3.8%	1.5%	1.0%
5	10.75µg ± 0.80	0.16µg ± 0.03	1.10µg ± 0.06	2.92µg ± 0.08	0.51µg ± 0.04	0.17µg ±0.01
	35.8%	0.5%	3.7%	9.7%	1.7%	0.6%
10	7.12µg ± 0.41	0.45µg ± 0.05	1.65µg ± 0.07	2.25µg ± 0.63	0.71µg ± 0.02	0.94µg ±0.14
	23.7%	1.5%	5.5%	7.5%	2.4%	3.1%
15	6.78µg ±0.53	0.4µg ±0.04	1.82µg ±0.08	3.09µg ±0.04	0.92µg ±0.07	0.85µg ±0.28
	22.6%	1.3%	6.1%	10.3%	3.1%	2.8%

Figure 25. Amount of propranolol remaining in the bloodstream and tissues following icv injection of 30 mcg radiolabelled propranolol in conscious New Zealand rats, expressed as micrograms (µg) (mean +/- standard error) and percentage of injected dose at times following icv injection.

Tissue / Time	Brain	Heart	Lungs	Liver	Kidneys	Blood
5	1.07µg ± 0.21	0.02µg ± 0.01	0.09µg ± 0.03	0.06µg ± 0.03	0.03µg ± 0.03	0.02µg ±0.01
	21.4%	0.3%	1.8%	1.2%	0.6%	0.4%
10	0.97µg ± 0.09	0.02µg ± 0.01	0.08µg ± 0.04	0.12µg ± 0.04	0.07µg ± 0.01	0.06µg ±0.01
	19.4%	0.3%	1.6%	2.4%	1.4%	1.2%
15	1.13µg ± 0.12	0.02µg ± 0.01	0.11µg ± 0.06	0.11µg ± 0.04	0.06µg ± 0.01	0.03µg ±0.01
	22.6%	0.4%	2.2%	2.2%	1.2%	0.6%
20	0.69µg ± 0.03	0.01µg ± 0.01	0.14µg ± 0.06	0.17µg ± 0.04	0.07µg ± 0.01	0.09µg ±0.01
	13.9%	0.2%	2.8%	3.4%	1.4%	1.7%

Figure 26. Amount of isoprenaline remaining in the bloodstream and tissues following icv injection of 5 mcg radiolabelled isoprenaline in conscious New Zealand rats, expressed as micrograms (µg) (mean +/- standard error) and percentage of injected dose at times following icv injection.

3.3.4. Discussion.

3.3.4.1. Leakage of drugs to the periphery following icv injection.

It was found that 10 to 15 minutes after icv injection of both isoprenaline and propranolol there was approximately 20% Of the injected dose remaining in the brain (see figures 25 and 26).

Anderson et al (1977) reported that, 10 minutes following icv injection of propranolol in conscious rabbits, plasma levels were 80% of those following iv injection of the same dose. This would produce peripheral cardiac beta-adrenoceptor blockade which would in turn confound any central component involved in the response to icv injection.

The rapid leakage to the peripheral bloodstream of icv injected propranolol in conscious spontaneously hypertensive rats was reported by Smits et al (1979). They showed that the plasma propranolol concentration-time curve was identical following subcutaneous or icv injection of 1 mg/Kg propranolol.

The evidence available suggests that, in conscious animals, bradycardia following icv injection of propranolol results

from blockade of cardiac beta- adrenoceptors, although the contribution of a central component cannot be excluded entirely.

Since the rate at which propranolol and isoprenaline leave the brain are similar, it appears that, as in anaesthetised animals, leakage occurs via bulk flow across the arachnoid villi and is independent of the lipophilicity of the drug (Schanker, 1962). Although large amounts of the drugs were observed to have leaked to the periphery, no large amounts in any one organ were detected. It is possible that significant amounts could have been taken up into subcutaneous fat or an alternative body compartment which had not been analysed in this study.

3.3.4.2. Icv and iv injection of propranolol.

In all animals injected with 30 mcg propranolol icv, hypotension and bradycardia were observed. Fifteen minutes following start of injection the extent of hypotension and bradycardia was 7 mmHg and 54 bpm.

Variable changes in mean arterial pressure following icv injection of propranolol in conscious animals have been reported by different authors. This study using rats agrees with the results of Day and Roach (1974a) who observed sustained falls in blood pressure in conscious

cats. However, most authors report an increase in arterial pressure. Conway and Lang (1974) observed a short lasting pressor response followed by a longer lasting hypotension after icv injection of 2 mg propranolol in conscious dogs. In conscious rabbits, an initial rise in mean arterial pressure followed by a hypotension at 4 hours was reported (Anderson et al, 1977; Dollery at al, 1973). This initial hypertension followed by hypotension at several hours following icv injection was also observed in conscious spontaneously hypertensive rats (Smits et al, 1979; Sweet and Wenger, 1976). It was suggested by Dollery et al (1973) that the initial increase in arterial pressure may be a result of the membrane stabilising action of propranolol, since it was mimicked by icv procaine.

In all studies involving the icv injection of propranolol into conscious animals, bradycardia was reported and this is consistent with the findings in this study.

Following intravenous injection of 30 mcg propranolol, a marked bradycardia (120 bpm) and a slight increase in mean arterial pressure (15 mmHg) were observed. It is not known whether propranolol would be taken up into the brain to any significant extent in the first 15 minutes following iv injection but it is likely that the bradycardia is a result of blockade of cardiac beta- adrenoceptors.

3.3.4.3. Effect of propranolol pretreatment on the responses to icv isoprenaline and clenbuterol.

Icv injection of isoprenaline produced a dose dependant hypotension and tachycardia (see figures 21, 22 and 23). The responses to 1 mcg isoprenaline icv were significantly reduced by 30 mcg propranolol icv, but the hypotension was unaffected by 30 mcg propranolol iv and the tachycardia was significantly potentiated. The potentiation of tachycardia to 1 mcg isoprenaline icv following pretreatment with 30 mcg propranolol iv was probably because the propranolol had reduced the baseline heart rate by over 100 bpm prior to injection of isoprenaline.

Day and Roach (1974b) reported variable changes in arterial pressure following icv injection of isoprenaline in conscious cats; whether pressor or depressor, these were abolished after icv injection of beta- adrenoceptor blocking agents. In all animals, tachycardia accompanied the change in blood pressure and this was also abolished by beta- adrenoceptor blocking agents.

Hypotension following icv isoprenaline in conscious rats was observed by Correa et al (1982) and Peres-Polon and Correa (1984). Pretreatment with phentolamine and phenoxybenzamine significantly potentiated the hypotension (Peres-Polon and Correa, 1984).

In this study, a propranolol insensitive mechanism involved in the response produced by icv isoprenaline did not appear to be present in the conscious rat, since pretreatment with icv propranolol abolished the hypotension and reduced the tachycardia. This mechanism may not be apparent in conscious animals if these responses are a result of a peripheral action of 'leaked' isoprenaline. However, a central action cannot be excluded since the hypotension caused by 1 mcg isoprenaline icv was reversed by icv, but not iv, propranolol.

Icv injection of clenbuterol caused hypotension and tachycardia, both significantly reduced by pretreatment with icv propranolol (see figures 24a and 24b). Nomura (1976) injected salbutamol into the lateral ventricle of conscious normotensive and hypertensive rats and observed an increase in blood pressure which was blocked by propranolol pretreatment but not by the beta1- adrenoceptor blocker, practolol. The pressor response was significantly increased in hypertensive animals, suggesting the involvement of beta2- adrenoceptors in the hypertensive state.

In conclusion, icv injection of drugs in conscious animals involves the major problem that large amounts of the injection appear to leak to the periphery in a short time,

suggesting that the responses may be mainly peripherally mediated, although a central component does seem to exist.

Icv injections in both anaesthetised and conscious animals have been shown to carry the implication that there is always the possibility of a peripheral action of 'leaked' drug masking any central action, and so in the next section an attempt to minimise this by injection directly into the hypothalamus has been made. It was hoped that by injection into discrete brain areas, it would be easier to attribute any cardiovascular responses to a central action of the injected drug.

3.4. Injection into the hypothalamus of anaesthetised New Zealand rats.

3.4.1. Injection of noradrenaline and the effect of pretreatment with propranolol. (2.2.5.)

Injection of noradrenaline into the anterior nucleus of the hypothalamus (anterior hypothalamus) resulted in an increase in blood pressure with no significant change in heart rate (see figures 27a and 27b). The maximum hypertension was 23 mmHg, achieved at 6 minutes following start of injection. Pretreatment with 30 mcg propranolol icv did not significantly alter the hypertension, but a significant ($p<0.05$) fall in heart rate occurred following injection of noradrenaline.

Injection of noradrenaline into the posterior nucleus of the hypothalamus (posterior hypothalamus) caused a fall in mean arterial pressure (12 mmHg) and an increase in heart rate of 57 bpm 20 minutes after start of injection (see figures 28a and 28b). Pretreatment with propranolol icv did not significantly alter the tachycardia, but significantly ($p<0.01$) reversed the hypotension to a hypertension of 16 mmHg occurring 1 minute after start of injection of noradrenaline.

3.4.2. Injection of adrenaline into the hypothalamus and pretreatment with propranolol. (2.2.5.)

Following injection of 5 mcg adrenaline into the anterior hypothalamus, a biphasic change in mean arterial pressure was observed. An initial increase of 4 mmHg was obtained 2 minutes following start of injection, this was followed by a longer-lasting fall in arterial pressure of 10 mmHg at 10 minutes. This was accompanied by a fall in heart rate of 40 bpm at 20 minutes (see figures 29a and 29b).

Injection of adrenaline into the posterior hypothalamus caused a fall in mean arterial pressure of 20 mmHg after 2 minutes and a bradycardia of 59 bpm after 8 minutes, both were returning to control levels 20 minutes after injection (see figures 30a and 30b). Pretreatment with 30 mcg propranolol icv significantly ($p<0.01$) reversed the hypotension to a hypertension of 25 mmHg and significantly ($p<0.05$) reduced the bradycardia.

3.4.3. Injection of clenbuterol into the hypothalamus and pretreatment with propranolol. (2.2.5.)

Injection of 5 mcg clenbuterol caused hypotension and tachycardia of similar magnitude whether injected into the anterior or posterior hypothalamus (see figures 31 and 32). Pretreatment with 30 mcg propranolol icv reduced the

hypotension produced by clenbuterol injected into the anterior hypothalamus, but this reduction did not reach statistical significance over the total duration of the experiment. The tachycardia produced by clenbuterol was potentiated by pretreatment with icv propranolol, but this did not reach statistical significance over the whole experiment (see figures 31a and 31b).

The hypotension produced by clenbuterol injected into the posterior hypothalamus was significantly ($p<0.05$) reduced by pretreatment with icv propranolol and had returned to the baseline value after 20 minutes (see figure 32a). The tachycardia was unaffected by propranolol pretreatment over the first 8 minutes, but then became significantly ($p<0.05$) potentiated (see figure 32b).

3.4.4. Injection of isoprenaline into the hypothalamus and pretreatment with beta- adrenoceptor blockers. (2.2.5. and 2.2.6.)

Injection of 5 mcg isoprenaline into the anterior or posterior hypothalamus produced a hypotension of 16 and 20 mmHg respectively. Accompanying this hypotension was a tachycardia of 21 and 15 bpm. Thus, the responses to intrahypothalamic isoprenaline were similar in magnitude whether injected into the anterior or posterior nuclei (see figs. 33 & 34).

Pretreatment with 30 mcg propranolol icv significantly ($p<0.05$) reduced the responses to isoprenaline injected into the anterior hypothalamus. Chronic oral dosing with 60 mg/Kg propranolol daily for 14 days significantly ($p<0.001$) reversed the hypotension and potentiated ($p<0.05$) the tachycardia (see figures 33a and 33b).

The magnitude of hypotension produced by isoprenaline injected into the posterior hypothalamus was not attenuated by pretreatment with icv propranolol, but the duration of hypotension was significantly reduced. The hypotension was significantly reduced ($p<0.05$) by chronic oral dosing with 60 mg/Kg propranolol daily for 14 days (see figure 34a). The tachycardia produced by isoprenaline injected into the posterior hypothalamus was significantly potentiated ($p<0.01$) by pretreatment with 60 mg/Kg propranolol daily for 14 days (see figure 334b).

The duration of hypotension produced by isoprenaline injected into the posterior hypothalamus was significantly reduced ($p<0.05$) by pretreatment with 30 mcg atenolol icv. The degree of tachycardia was significantly potentiated ($p<0.01$) by atenolol pretreatment (see figures 35a and 35b).

Pretreatment with 30 mcg ICI 118,551 icv significantly potentiated the hypotension at 2 minutes ($p<0.01$), but the

hypotension quickly returned to a similar degree as that seen in non-pretreated animals. Tachycardia to isoprenaline was significantly potentiated by ICI 118,551 ($p<0.05$) over the first 6 minutes of the experiment (see figures 36a and 36b).

In animals dosed orally with 50 mg/Kg atenolol daily for 7 days, injection of isoprenaline into the anterior hypothalamus caused a greater degree of hypotension than in untreated animals (see figure 37a). Pretreatment with 30 mcg propranolol icv significantly reduced this hypotension ($p<0.01$). The tachycardia produced by isoprenaline in atenolol predosed rats was similar to that in untreated animals, and further pretreatment with icv propranolol did not significantly alter the degree of tachycardia (see figure 37b).

Injection of propranolol into the anterior hypothalamus caused a small biphasic change in mean arterial pressure accompanied by a large bradycardia of over 100 bpm (see figures 38a and 38b). Administration of isoprenaline and propranolol into the anterior hypothalamus in a single injection caused an initial sharp fall in mean arterial pressure followed by a longer lasting increase in pressure. This did not appear to be the result of simple addition of the effects of the two drugs (see figure 38a). A biphasic change in heart rate was also observed, and this was a

closer approximation to a summation of the two drugs acting independently (see figure 38b).

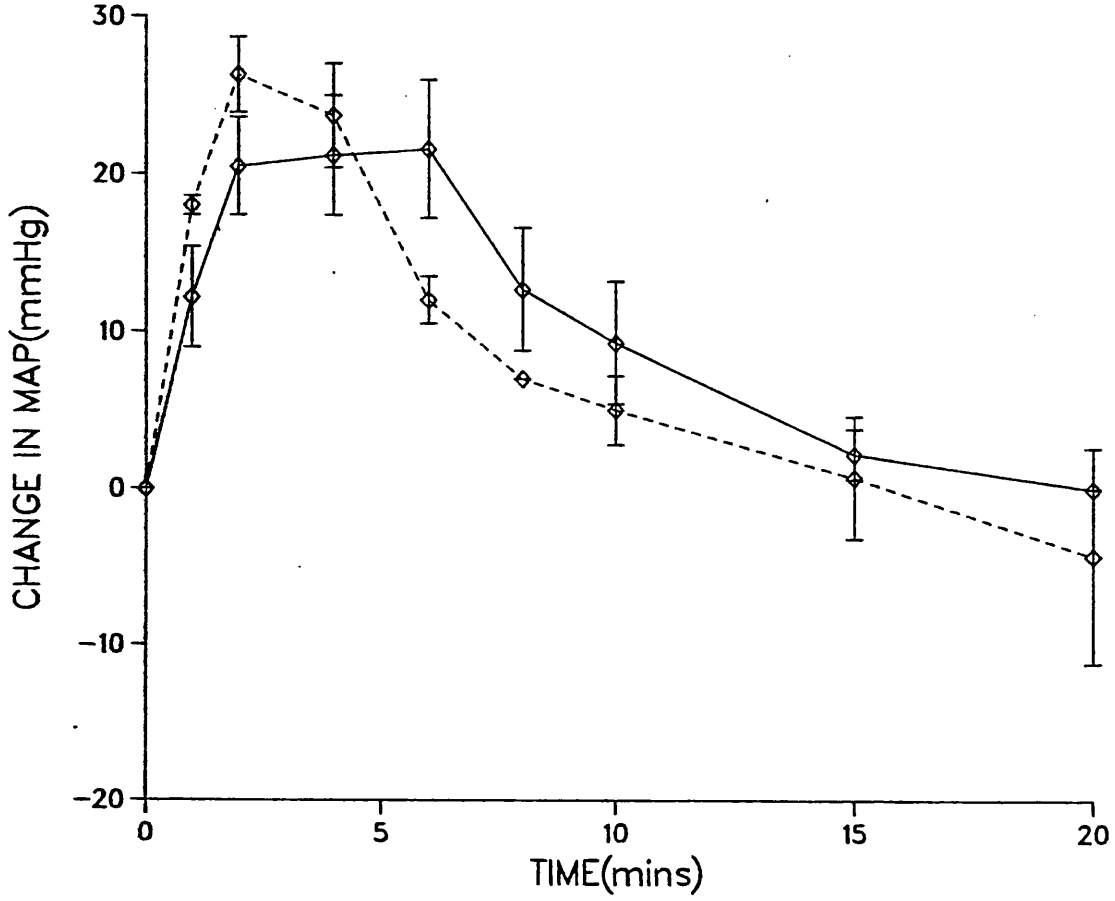

Figure 27a.

Figures 27a and 27b. Change in mean arterial pressure and heart rate produced by 5 mcg noradrenaline injected into the anterior hypothalamus of anaesthetised New Zealand rats.

◇————◇ No pretreatment (n=6) 94 mmHg, 465 bpm.

◇- - - - -◇ 30 mcg propranolol icv (n=6) 100 mmHg, 390 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$

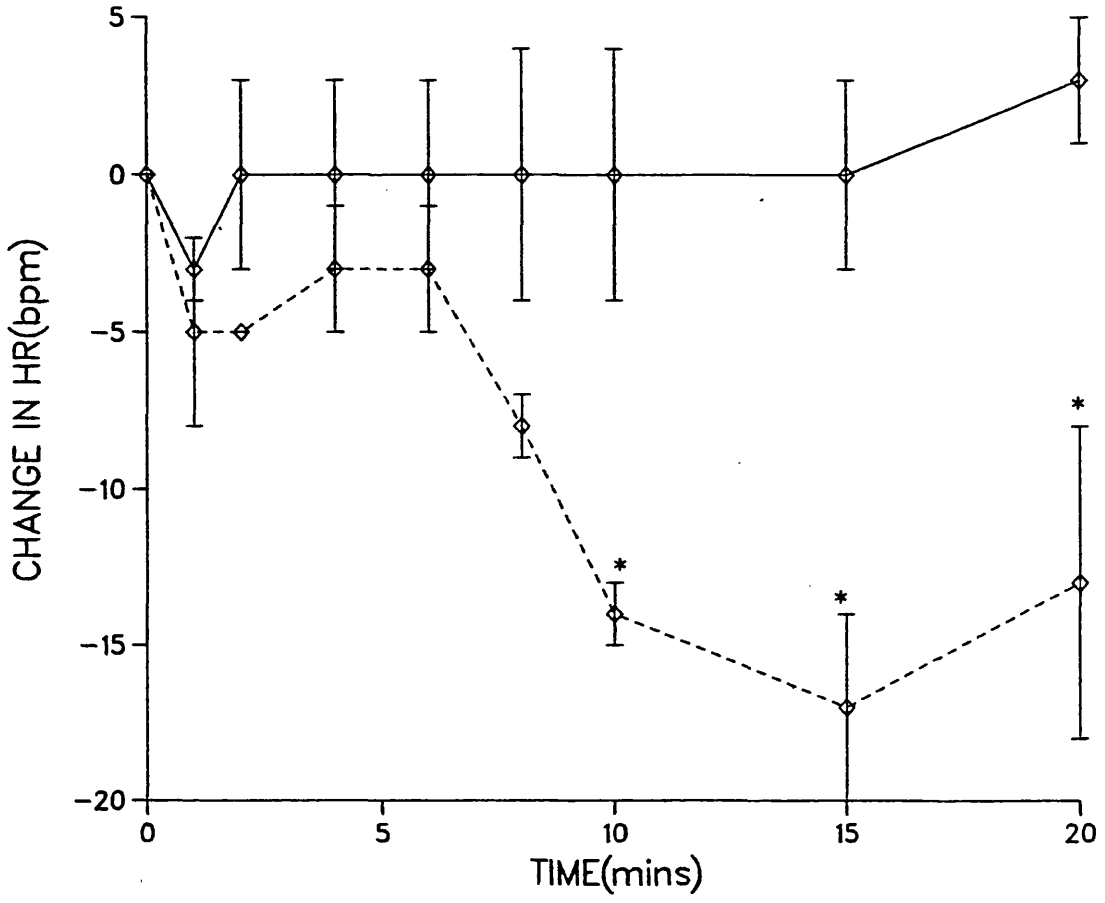

Figure 27b.

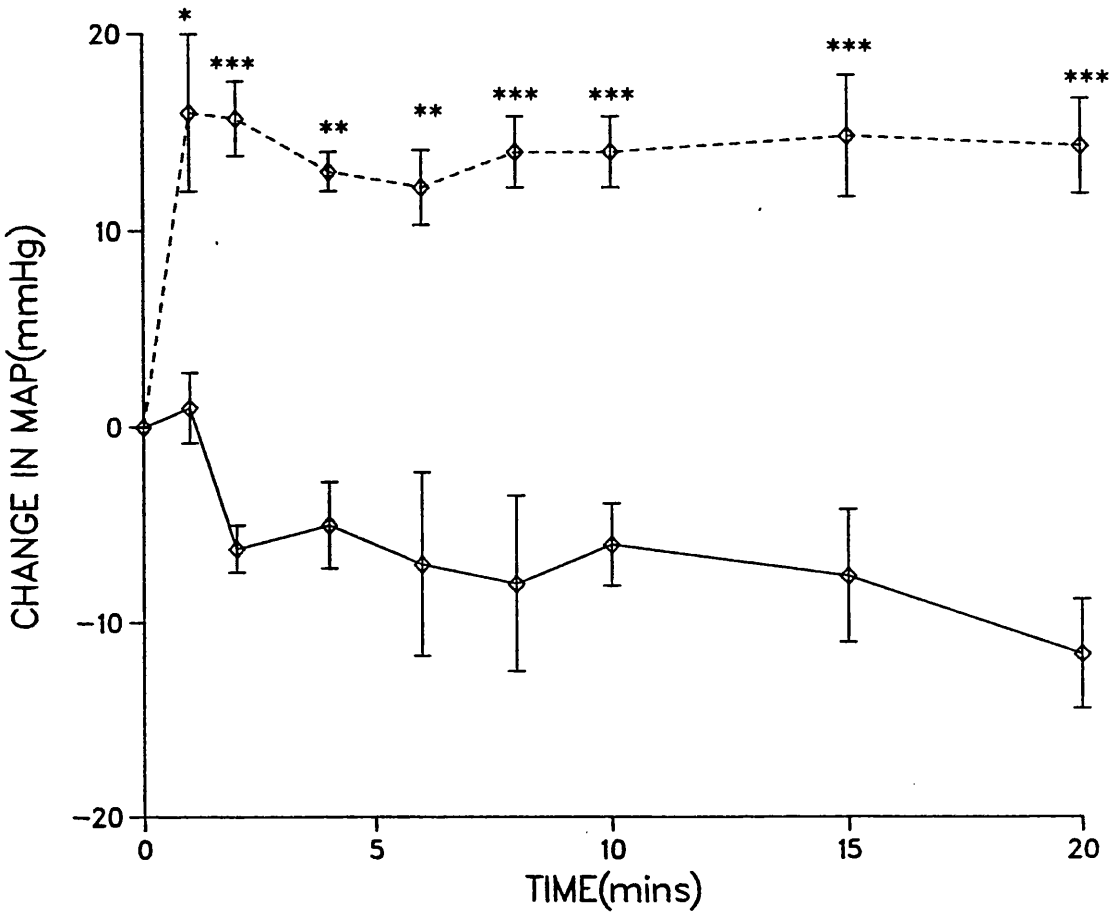

Figure 28a.

Figures 28a and 28b. Change in mean arterial pressure and heart rate produced by 5 mcg noradrenaline injected into the posterior hypothalamus of anaesthetised New Zealand rats.

◇——————◇ No pretreatment (n=6) 105 mmHg, 440 bpm.

◇- - - - - -◇ 30 mcg propranolol icv (n=6) 97 mmHg, 343 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

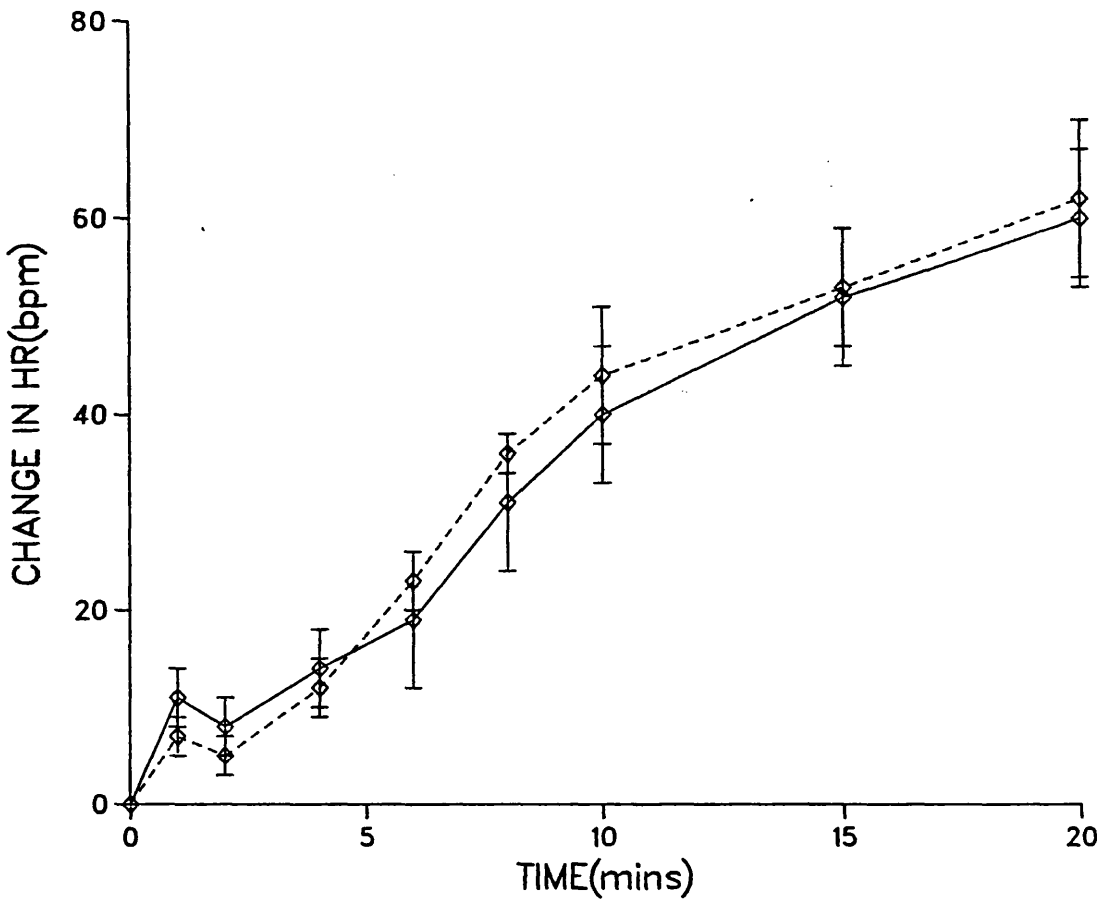

Figure 28b.

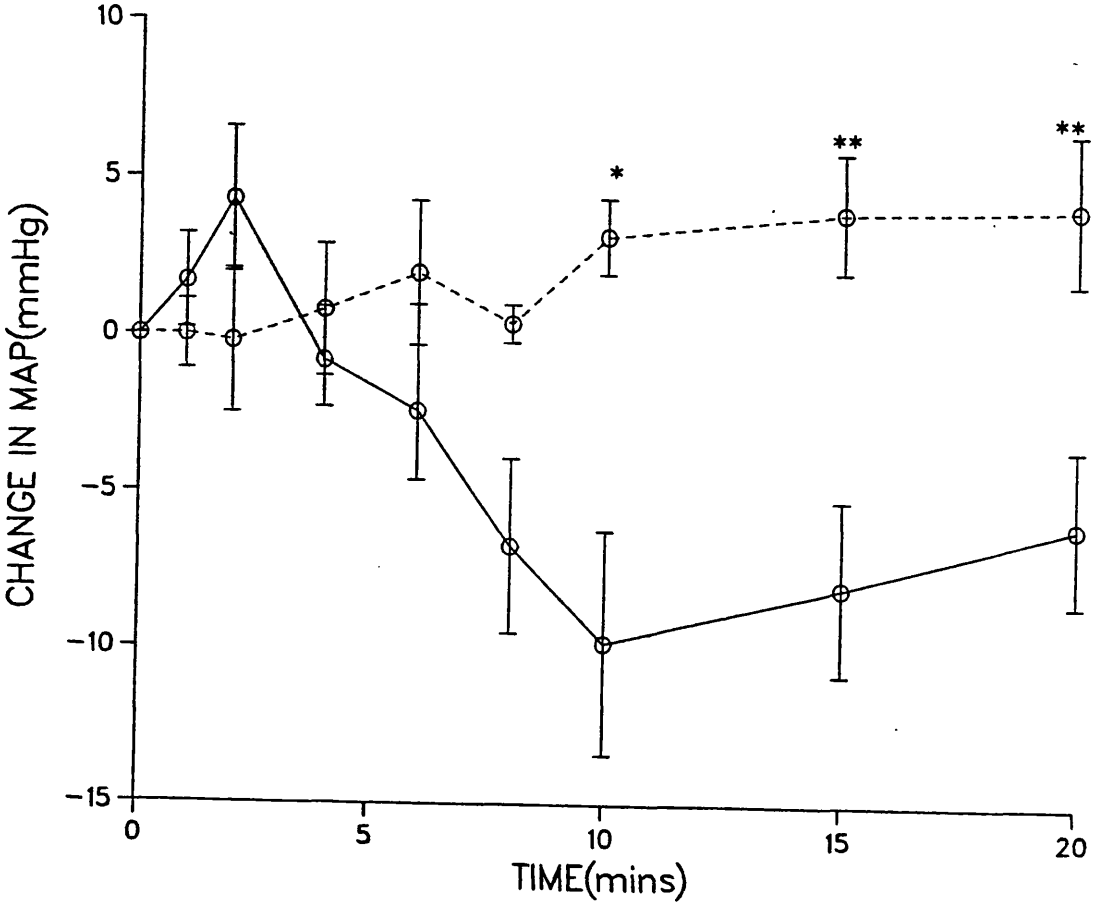

Figure 29a.

Figures 29a and 29b. Change in mean arterial pressure and heart rate produced by 5 mcg adrenaline injected into the anterior hypothalamus of anaesthetised New Zealand rats.

o———o No pretreatment (n=12) 76 mmHg, 443 bpm.

o- - - - - - -o 30 mcg propranolol icv (n=6) 87 mmHg, 342 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

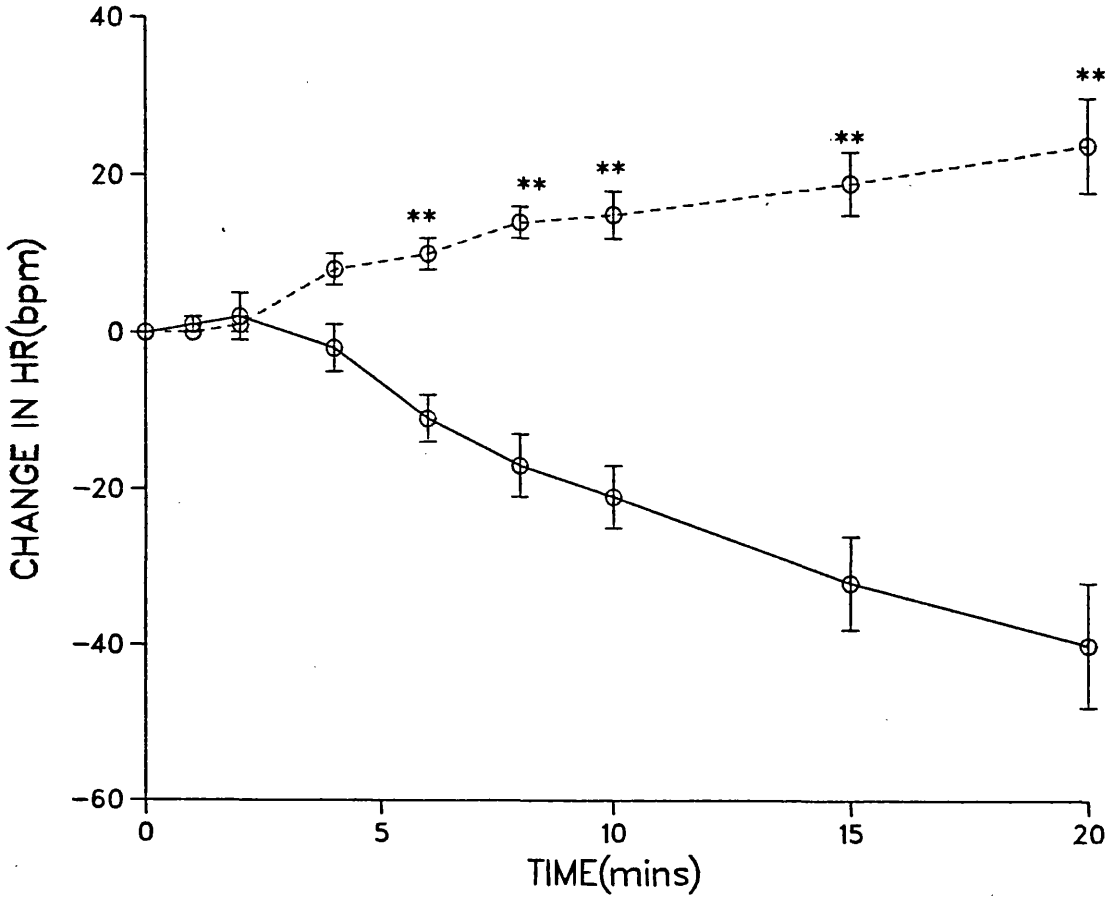

Figure 29b.

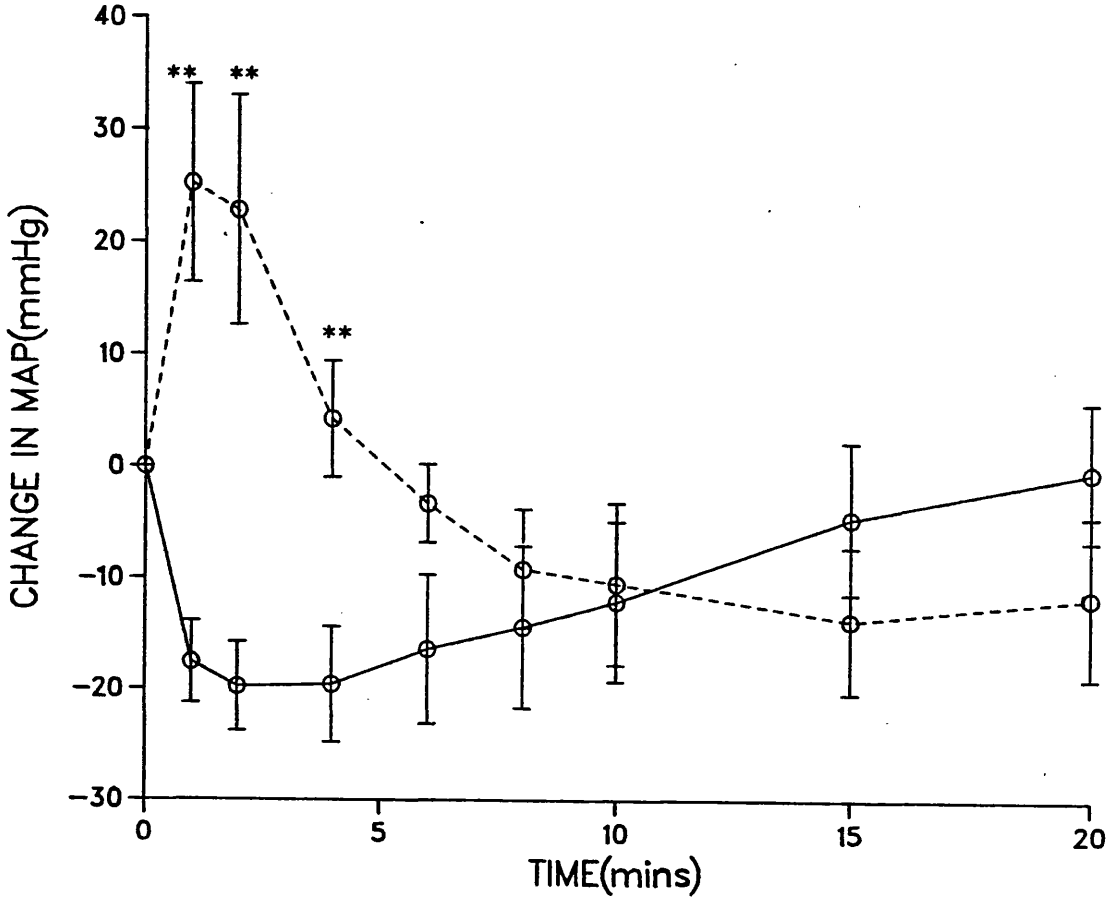

Figure 30a.

Figures 30a and 30b. Change in mean arterial pressure and heart rate produced by 5 mcg adrenaline injected into the posterior nucleus of the hypothalamus in anaesthetised New Zealand rats.

⊙———⊙ No pretreatment (n=8) 79 mmHg, 447 bpm.

⊙--------⊙ 30 mcg propranolol icv (n=8) 126 mmHg, 366 bpm

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

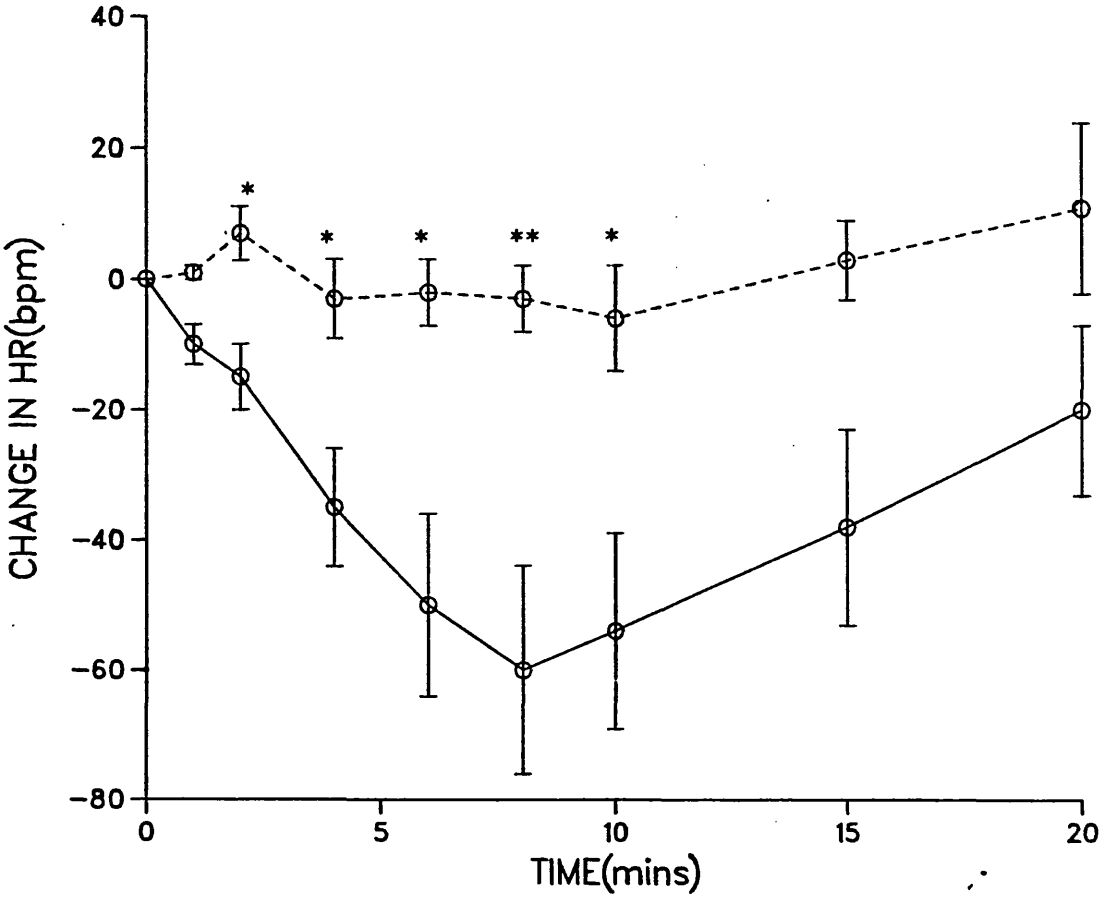

Figure 30b.

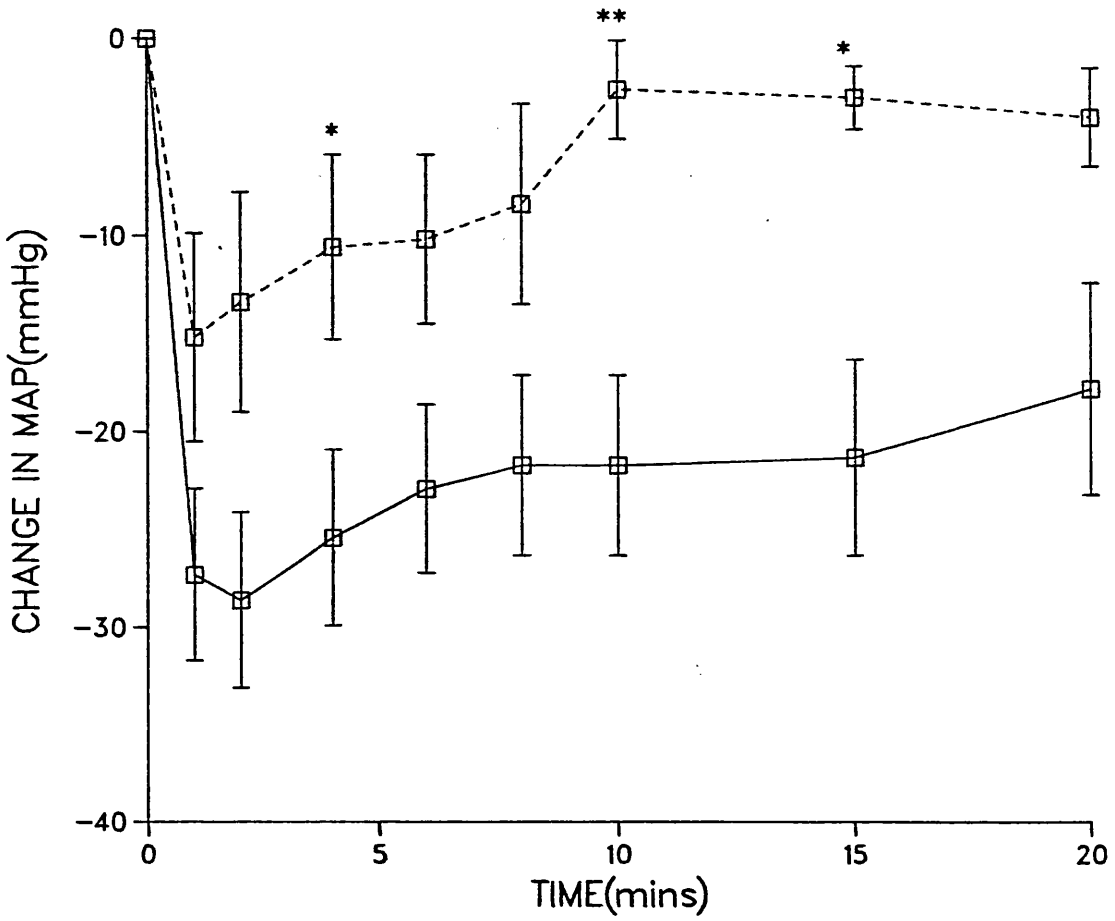

Figure 31a.

Figures 31a and 31b. Change in mean arterial pressure and heart rate produced by 5 mcg clenbuterol injected into the anterior hypothalamus in anaesthetised New Zealand rats.

No pretreatment (n=7) 87 mmHg, 420 bpm.

30 mcg propranolol icv (n=6) 87 mmHg, 395 bpm

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

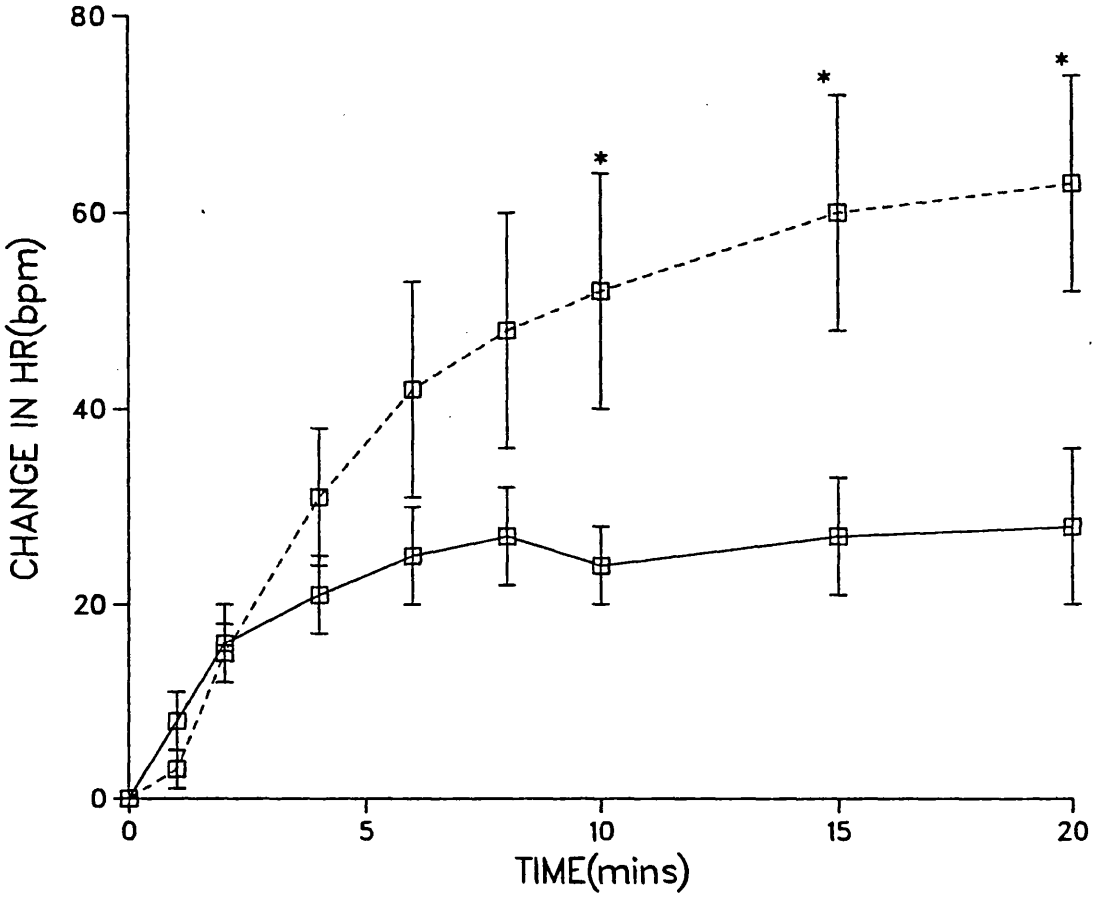

Figure 31b.

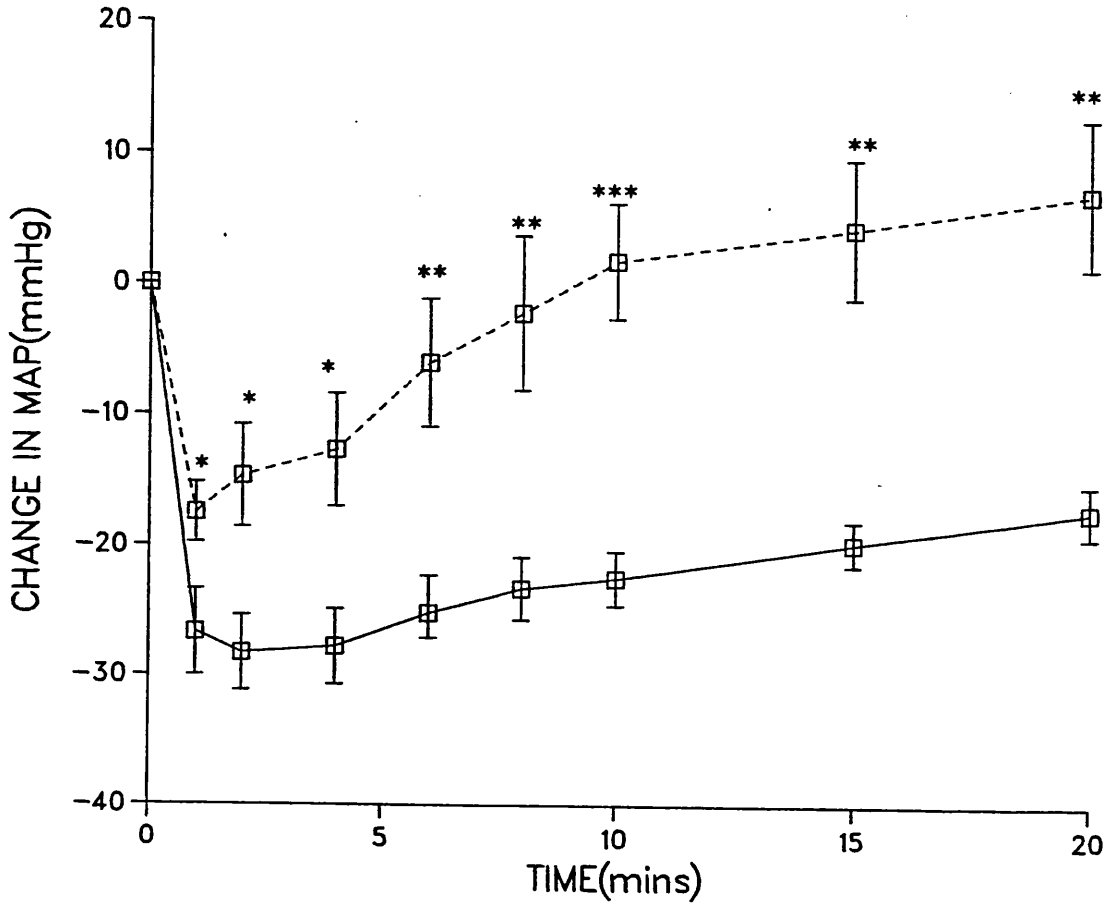

Figure 32a.

Figures 32a and 32b. Change in mean arterial pressure and heart rate produced by 5 mcg clenbuterol injected into the posterior hypothalamus in anaesthetised New Zealand rats.

No pretreatment (n=6) 75 mmHg, 455 bpm.

30 mcg propranolol icv (n=6) 77 mmHg, 390 bpm

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

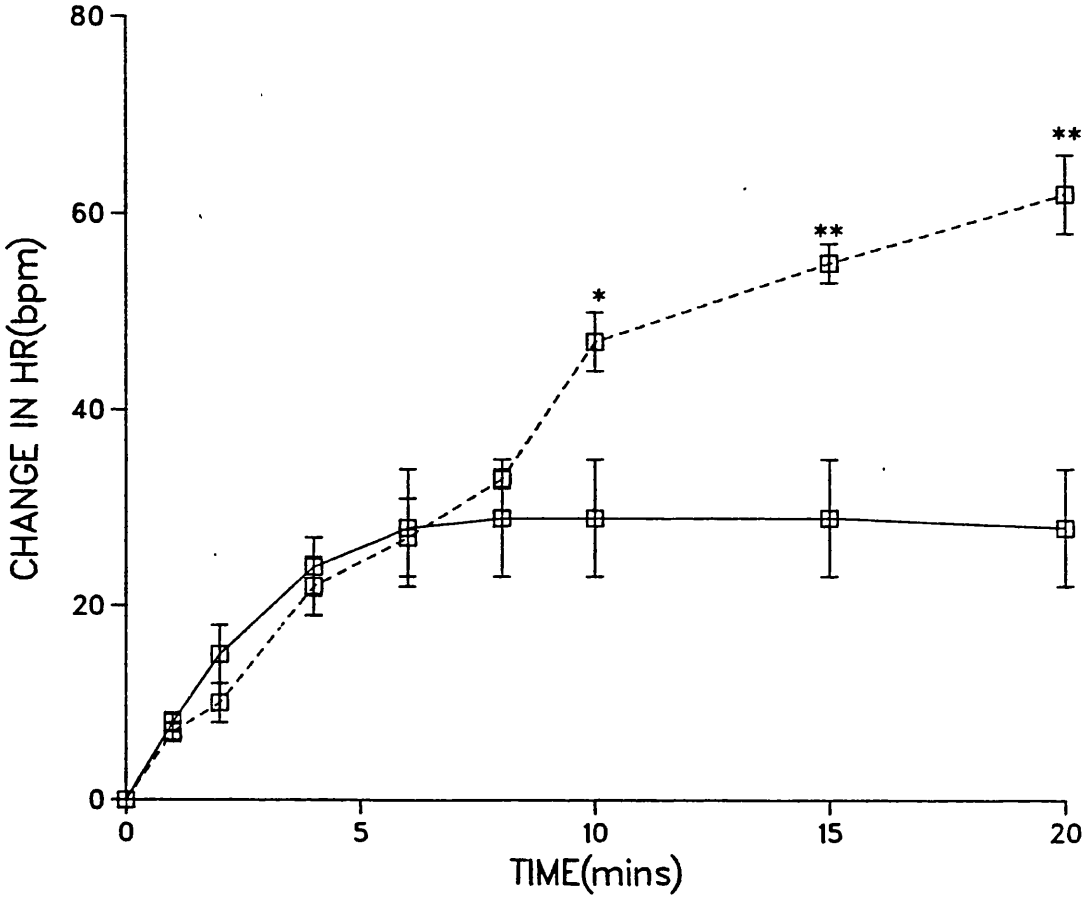

Figure 32b.

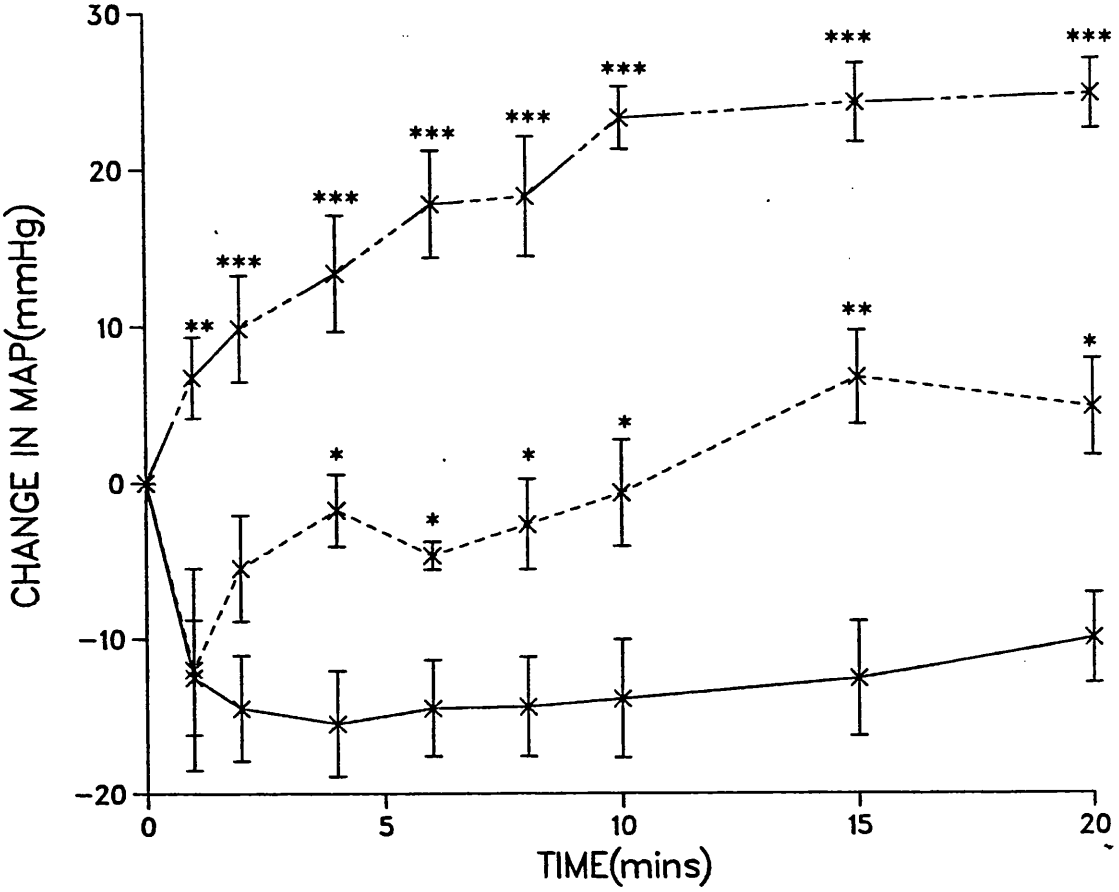

Figure 33a.

Figures 33a and 33b. Change in mean arterial pressure and heart rate produced by 5 mcg isoprenaline injected into the anterior hypothalamus in anaesthetised New Zealand rats.

x————x No pretreatment (n=11) 79 mmHg, 432 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 108 mmHg, 368 bpm

x—— - - -——x 60 mg/Kg propranolol po daily for 14 days (n=6) 71 mmHg, 318 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

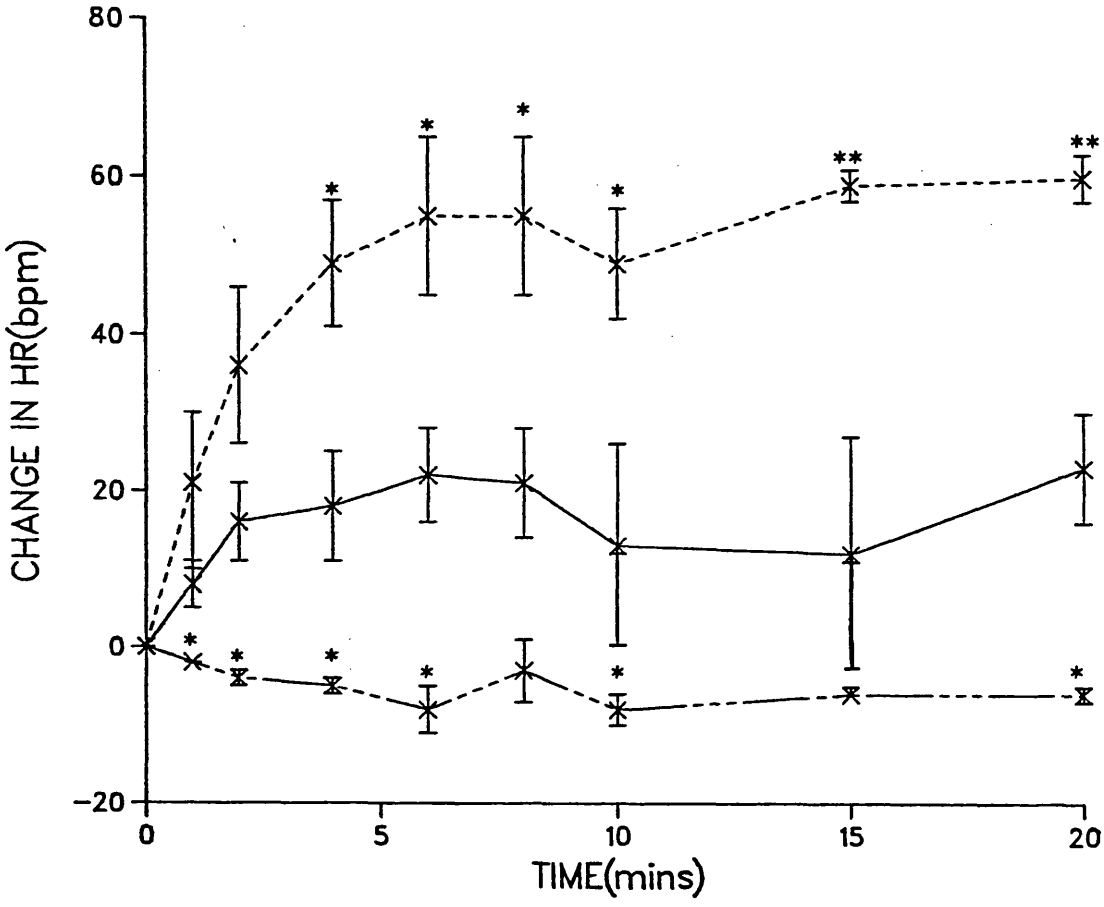

Figure 33b.

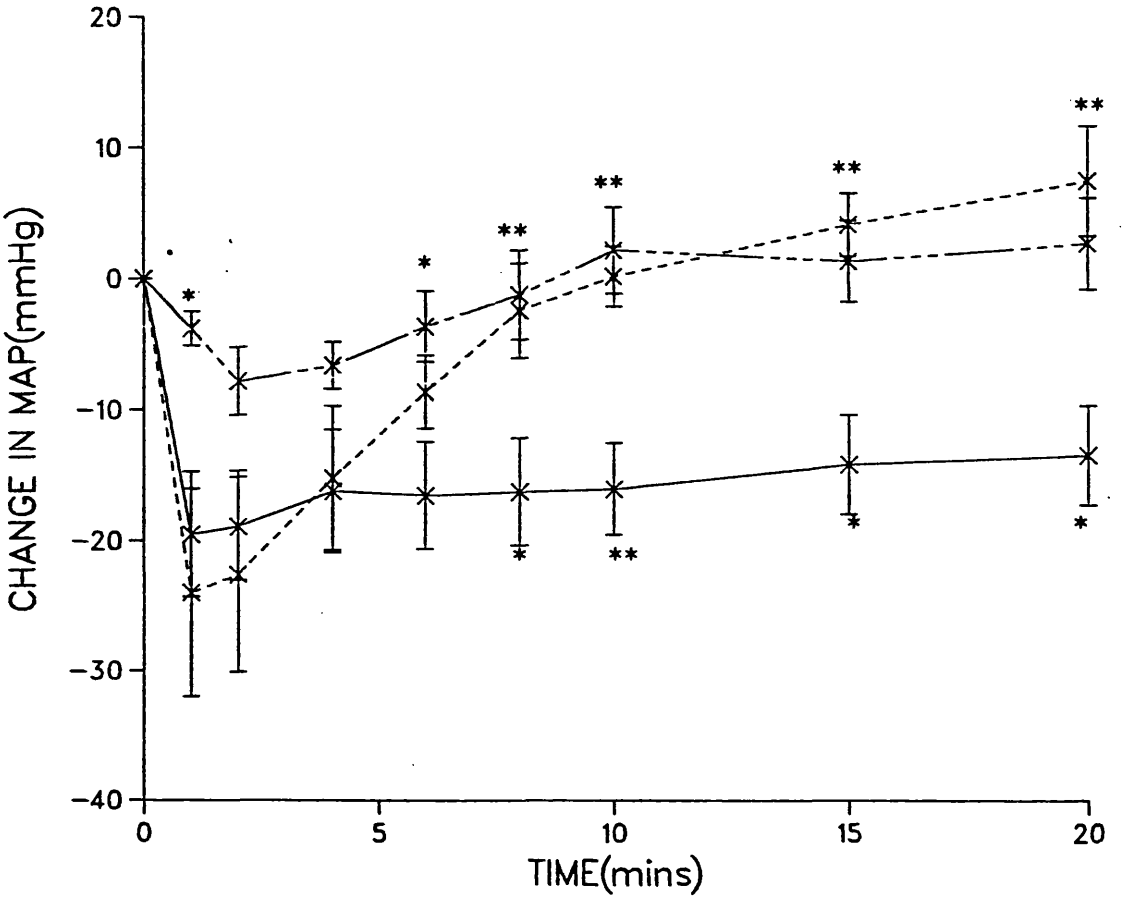

Figure 34a.

Figures 34a and 34b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the posterior hypothalamus of anaesthetised New Zealand rats.

x———x No pretreatment (n=8) 76 mmHg, 422 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=5) 103 mmHg, 401 bpm.

x—— ·-·-·—x 60 mg/Kg propranolol po daily for 14 days (n=5) 112 mmHg, 305 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

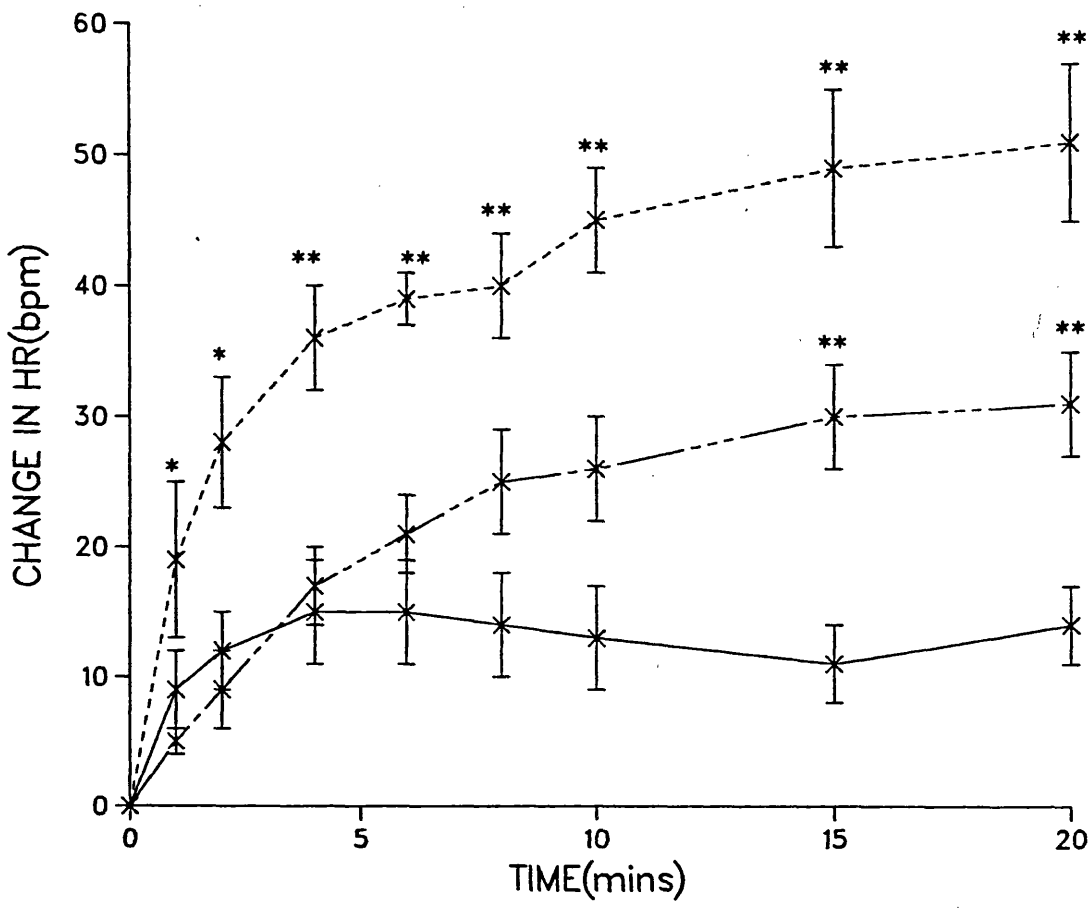

Figure 34b.

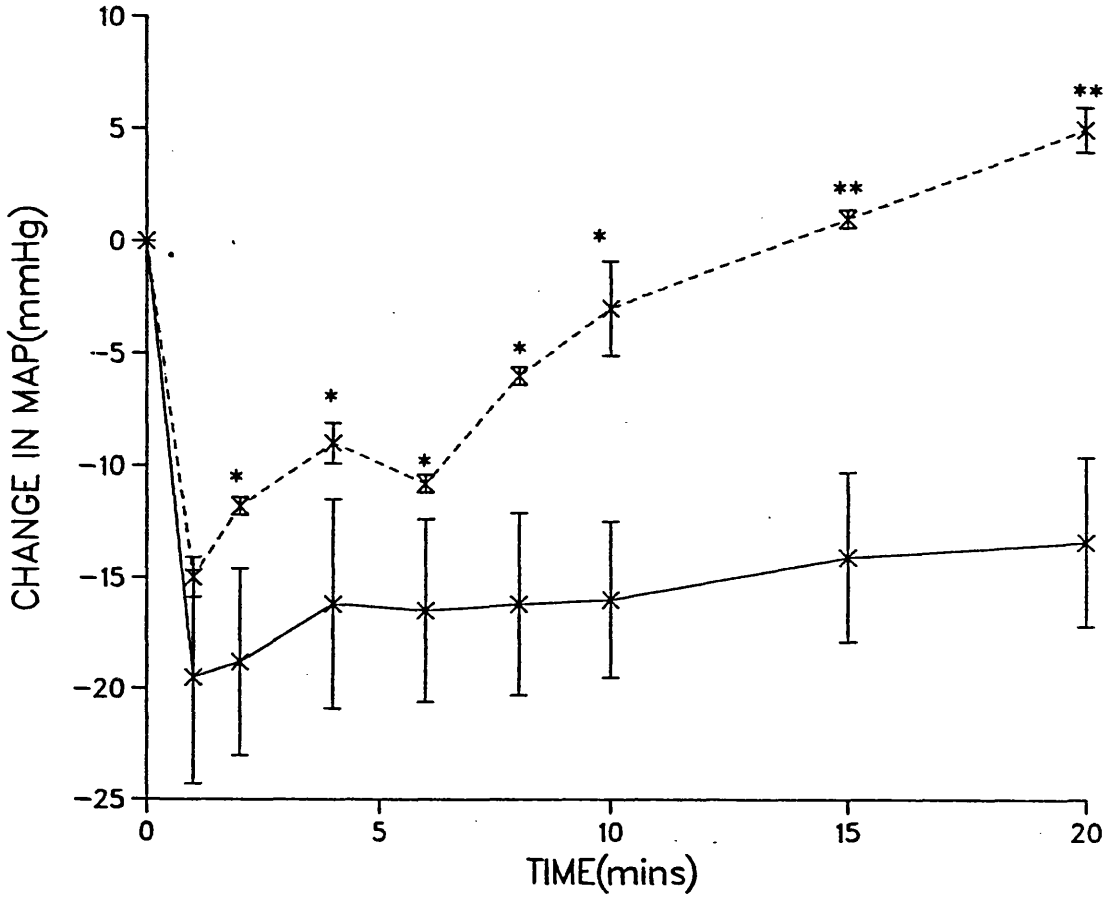

Figure 35a.

Figures 35a and 35b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the posterior hypothalamus of anaesthetised New Zealand rats.

x————x No pretreatment (n=8) 76 mmHg, 422 bpm.

x- - - - - -x 30 mcg atenolol icv (n=6) 64 mmHg, 465 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

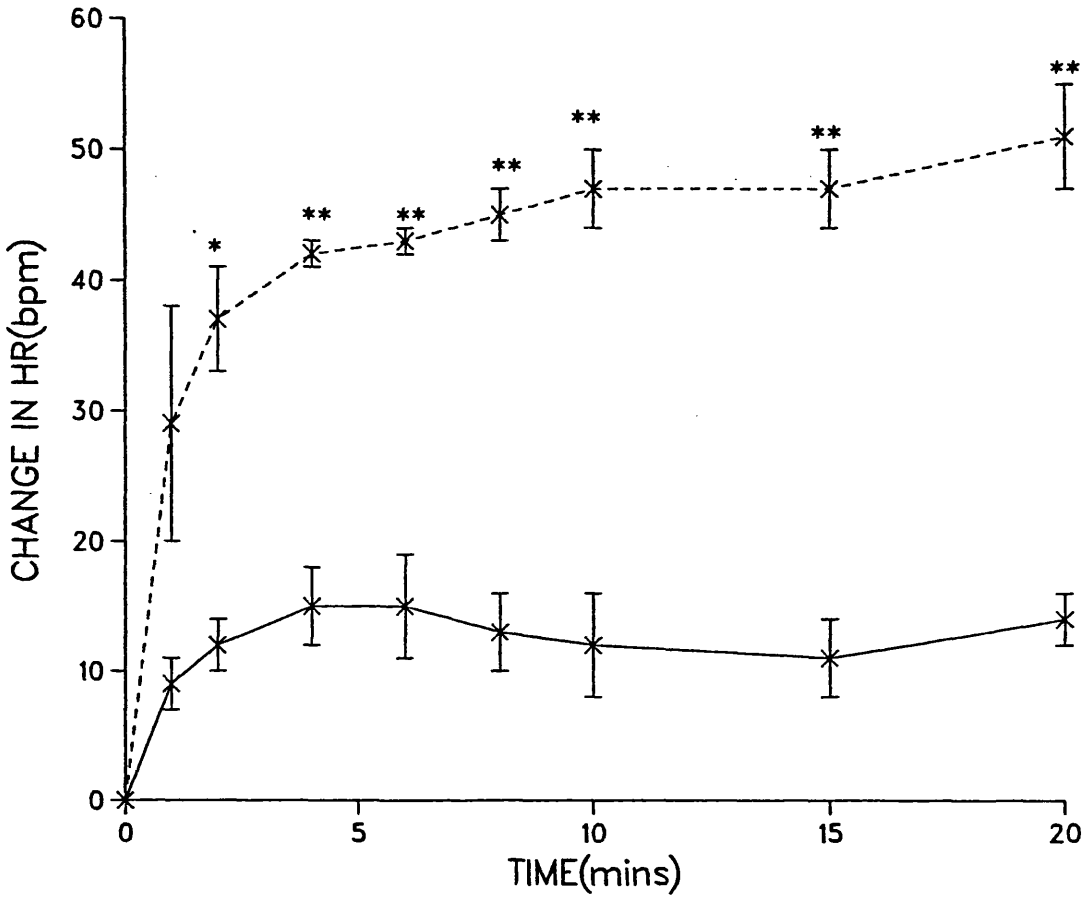

Figure 35b.

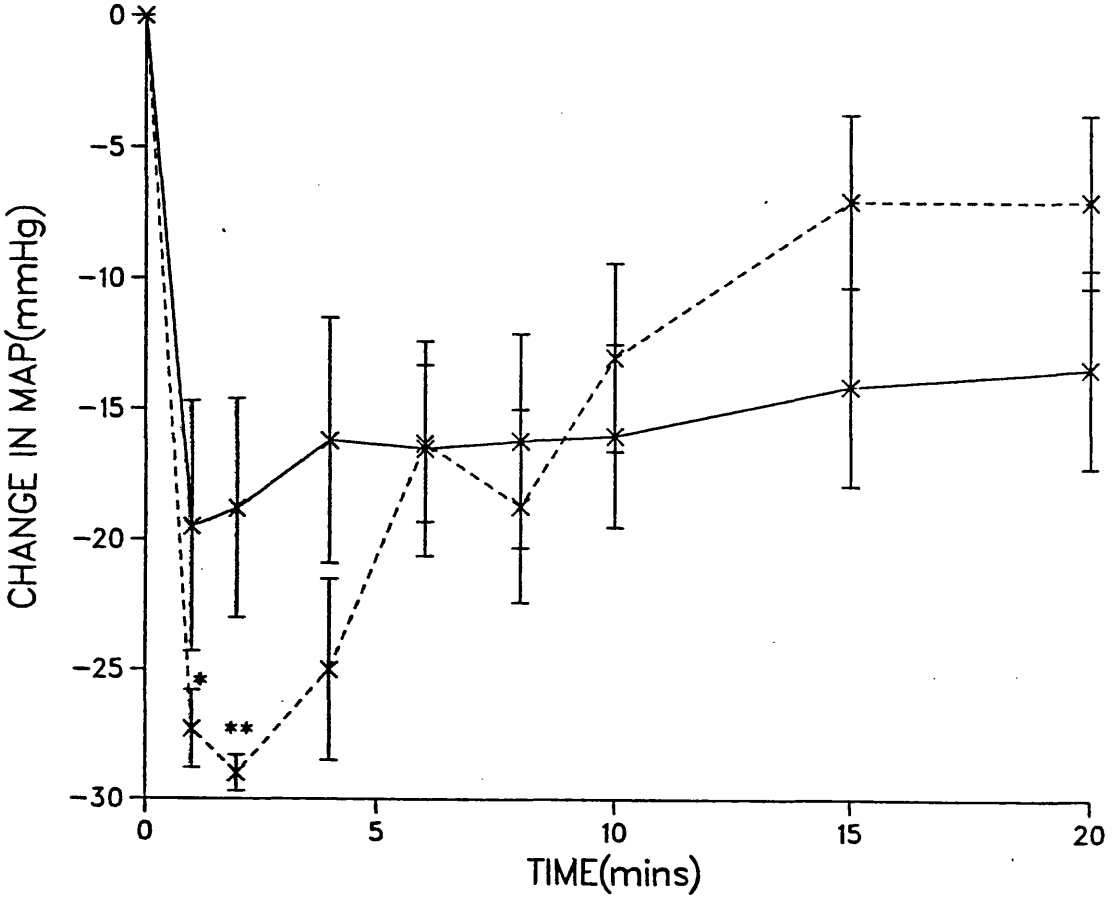

Figure 36a.

Figures 36a and 36b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the posterior hypothalamus of anaesthetised New Zealand rats.

x————x No pretreatment (n=8) 76 mmHg, 422 bpm.

x- - - - - -x 30 mcg ICI 118,551 icv (n=6) 106 mmHg, 496 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$

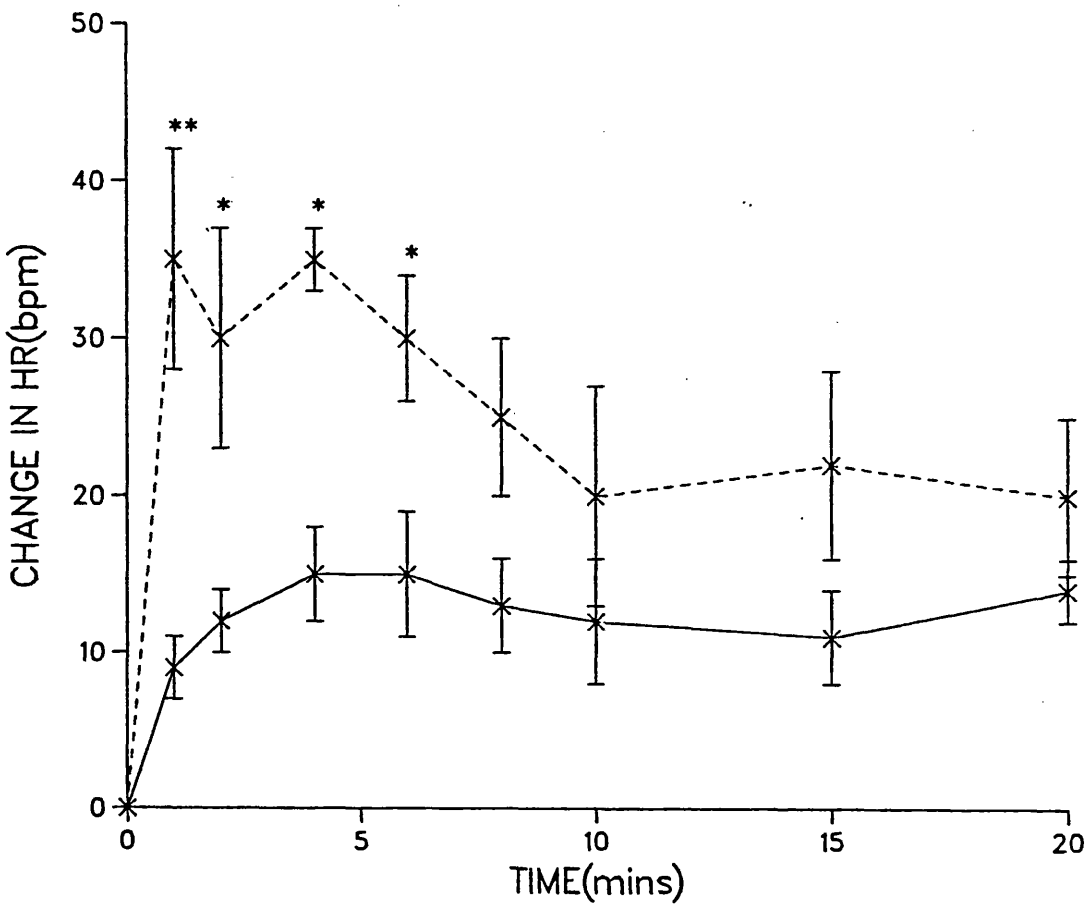

Figure 36b.

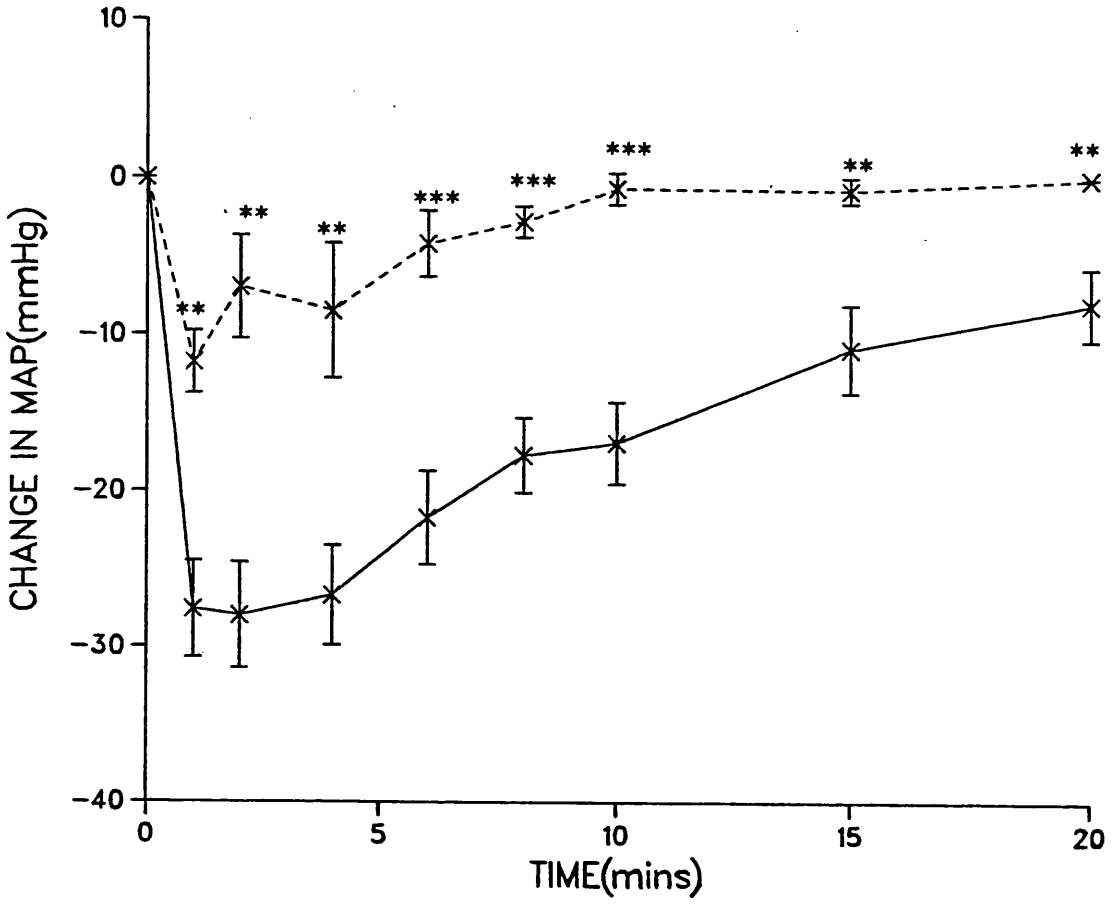

Figure 37a.

Figures 37a and 37b. Change in mean arterial pressure and heart rate produced by 5 mcg isoprenaline injected into the anterior hypothalamus of anaesthetised New Zealand rats dosed orally with 50 mg/kg atenolol daily for 7 days.

x———x No pretreatment (n=6) 102 mmHg, 300 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 110 mmHg, 294 bpm.

Significant difference from no pretreatment group denoted:

** $p<0.01$ *** $p<0.001$

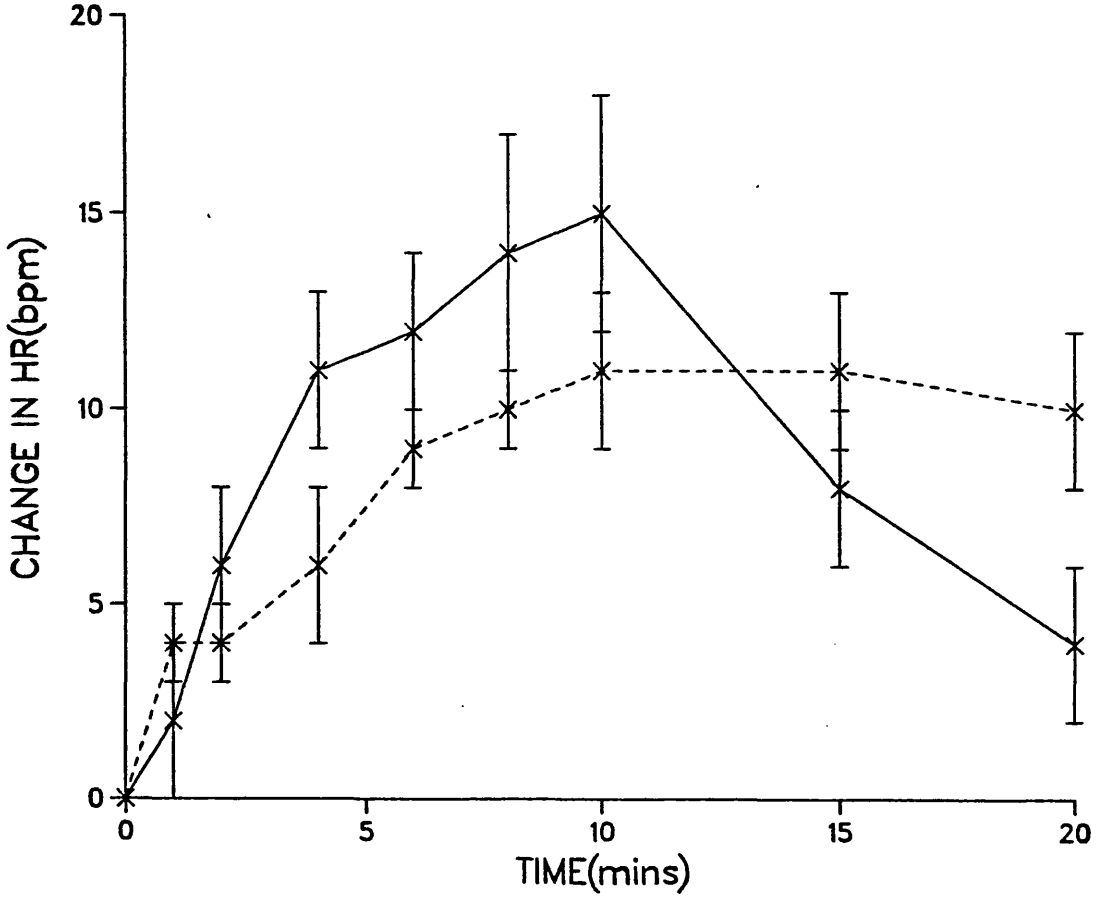

Figure 37b.

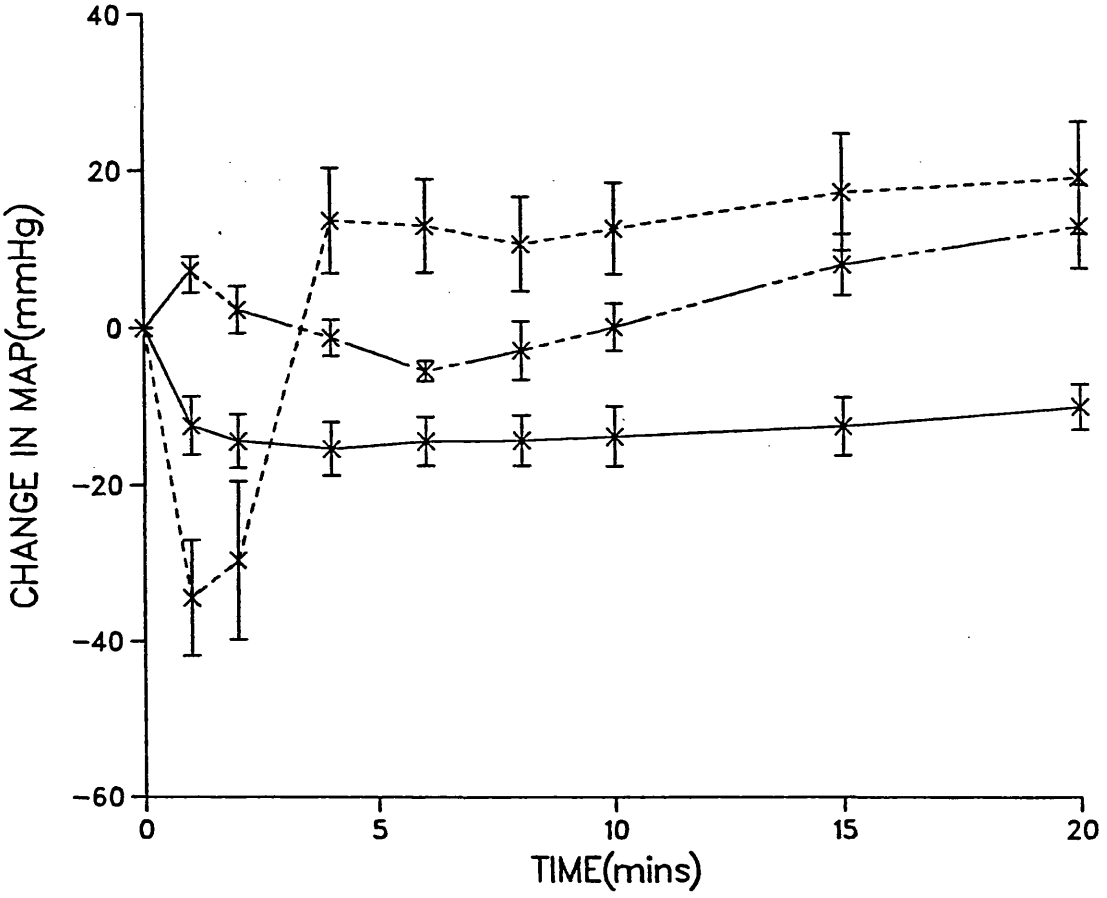

Figure 38a.

Figures 38a and 38b. Change in mean arterial pressure and heart rate following injection into the anterior hypothalamus of anaesthetised New Zealand rats.

x————x 5 mcg isoprenaline (n=11) 79 mmHg, 432 bpm.

x————- - - -x 30 mcg propranolol (n=6) 108 mmHg, 469 bpm.

x- - - - - -x 30 mcg propranolol and 5 mcg isoprenaline (n=6) 114 mmHg, 446 bpm.

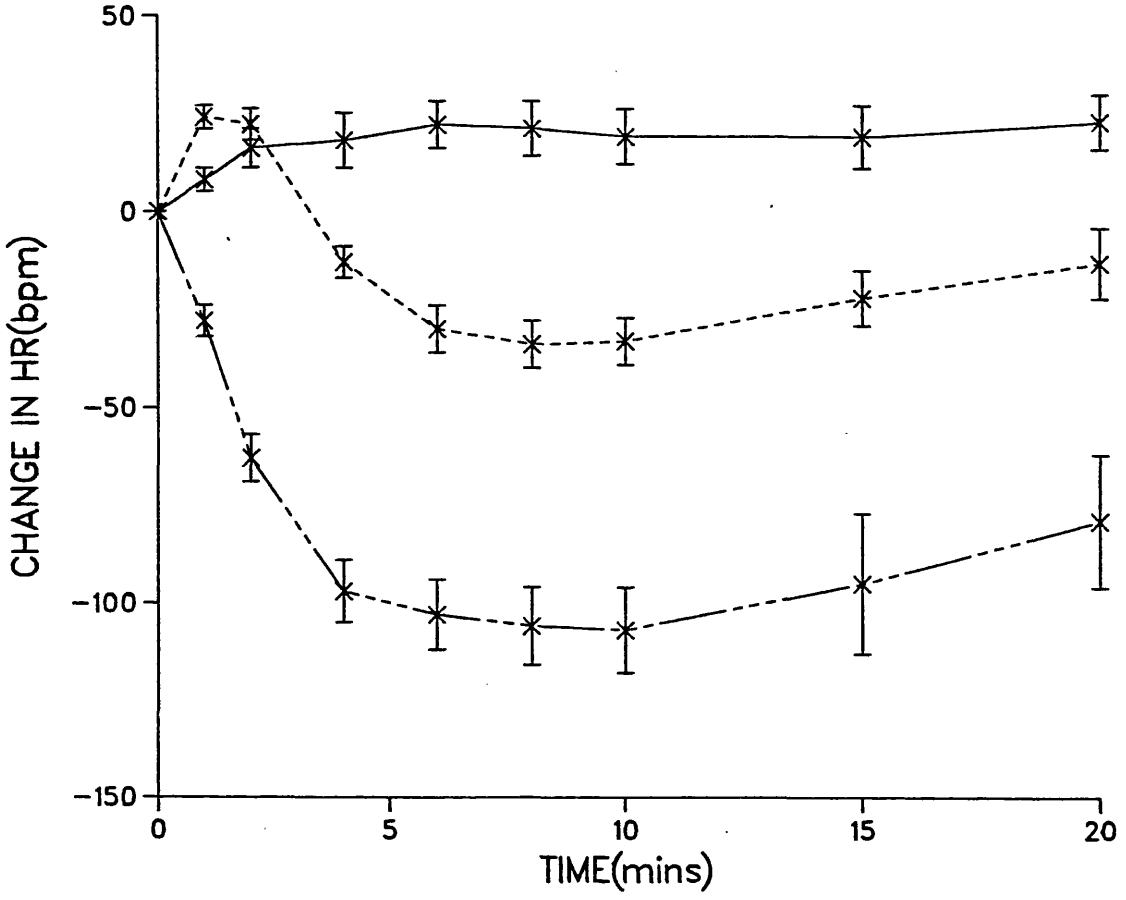

Figure 38b.

Figure 38c. Spread of Evans Blue dye through brain tissue following injection of 1 mcl into the anterior hypothalamus.

3.4.5. Discussion.

The problems associated with differentiating between central and peripheral effects following injection into the cerebral ventricle led to consideration of injections into discrete areas of the brain. It was hoped that during the course of the experiment insufficient drug would leak into the periphery to exert a peripheral effect. Localised injection would also serve to localise the possible sites of action within the central nervous system at which drugs acting at beta- adrenoceptors may exert an effect.

Following peripheral administration of propranolol, significant levels have been detected in the hypothalamus of rabbits, cats, dogs and rats (Bakke et al, 1974; Elghozi et al, 1979; Garvey and Ram, 1975), suggesting the presence of beta- adrenoceptors in the hypothalamus.

The hypothalamus, amygdala and hippocampus appear to control the activity of the nuclei in the medulla oblongata and belonging to the baroreceptor reflex loop. Direct connections to the medulla and spinal cord have been demonstrated from various nuclei of the hypothalamus, including the posterior and lateral hypothalamic areas and dorsomedial and paraventricular nuclei (Loewy and McKellar, 1980).

The hypothalamus is implicated as an area within the central nervous system which regulates cardiovascular function and it was decided to observe any changes in cardiovascular parameters following direct injection into the hypothalamus.

Injections were made into the anterior and posterior nuclei of the hypothalamus because Gellhorn (1964) postulated the presence of two distinct autonomic areas within the hypothalamus, the anterior portion containing the parasympathetic centre and the sympathetic centre being located posteriorly. There appear to be conflicting effects upon cardiovascular parameters depending on whether injections are made into the anterior or posterior hypothalamus, and it was hoped to illustrate any differences in this study.

3.4.5.1. Injection of noradrenaline into the hypothalamus and pretreatment with propranolol.

Injection of noradrenaline into the anterior hypothalamus elicited an increase in mean arterial pressure with no significant change in heart rate. Pretreatment with icv propranolol did not significantly alter the hypertension but did produce a bradycardia following injection of noradrenaline (see figures 27a and 27b). In anaesthetised rats, Struyker Boudier et al (1974) reported a fall in

blood pressure and heart rate in response to the injection of 40 nmol noradrenaline into the anterior hypothalamus, but a transient increase in blood pressure following the injection of 100 nmol noradrenaline. They suggested that the increase in blood pressure following the injection of the larger dose of noradrenaline was probably due to vascular transport of small amounts of noradrenaline to the peripheral circulation where it can induce hypertension in very low concentrations. The depressor response was thought to be a result of stimulation of hypothalamic alpha- adrenoceptors by noradrenaline since bradycardia and hypotension were induced by phenylephrine and were antagonised by phentolamine.

Although hypertensive responses to noradrenaline were obtained in this study, it is thought that this was a result of an action upon central alpha- adrenoceptors since the responses was not antagonised by pretreatment with propranolol. Since the maximum response was observed 2 minutes following the start of injection, it was thought unlikely that significant amounts would have leaked to the periphery from the hypothalamus in this short time.

Following injection of noradrenaline into the posterior hypothalamus, hypotension and tachycardia were observed. Pretreatment with propranolol significantly reversed the hypotension, but had no effect on the tachycardia.

Struyker Boudier et al (1974) found no change in cardiovascular parameters following injection of noradrenaline into the posterior hypothalamus. Since the hypotension observed in this study was significantly altered by pretreatment with propranolol, it is possible that this response could arise from an interaction of noradrenaline with beta- adrenoceptors in the posterior hypothalamus. However, Philippu and Kittel (1977) have demonstrated that activation of beta- adrenoceptors in the posterior hypothalamus is likely to elicit increases in arterial pressure.

Philippu et al (1971) observed dose dependant increases in blood pressure following superfusion of the hypothalamus of anaesthetised cats with noradrenaline; this effect was strongly diminished by transection of the spinal cord. The response to noradrenaline was found to be resistant to phentolamine, but at higher doses this drug caused ventricular haemorrhage which made interpretation of results difficult.

Electrical stimulation of the anterior hypothalamus has shown varying changes in arterial pressure in different species. Decreases in pressure have been observed in dogs (Bogaert et al, 1976) cats (Eliasson et al, 1951) and rats (Gamble and Patton, 1953) whereas increases in pressure have been reported in rats (Faiers et al, 1976).

Although the cardiovascular changes observed following electrical stimulation are almost invariably due to neural effector mechanisms, Calaresu and Ciriello (1979) reported an increase in arterial pressure following stimulation of the paraventricular nucleus which could be due to a humoral mechanism. They suggested that the response may be a result of the release of vasopressin.

3.4.5.2. Injection of adrenaline into the hypothalamus and pretreatment with propranolol.

Struyker Boudier and Bekers (1975) injected adrenaline into the anterior hypothalamus of anaesthetised rats and observed a dose dependant decrease in arterial pressure and heart rate. A small, but significant, rise in arterial pressure was observed immediately after injection, this did not depend on the dose of adrenaline injected.

In this study, 5 mcg adrenaline caused a biphasic change in mean arterial pressure with bradycardia (see figures 29a and 29b), these findings agree with those of Struyker Boudier and Bekers (1975). Pretreatment with icv propranolol did not significantly alter the initial increase in mean arterial pressure, but did abolish the hypotension which occurred 10 minutes following injection. The bradycardia was also significantly blocked by

propranolol. These findings suggest that the hypotension and bradycardia may be mediated via beta- adrenoceptors whereas the initial transient increase in pressure is not. Struyker Boudier and Bekers (1975) suggested that this increase in pressure was probably a result of leakage of some adrenaline into the peripheral circulation. However, it is unlikely that a leakage to the periphery from the hypothalamus would occur only 2 minutes following injection and, if so, it is equally likely that leakage would continue over the whole duration of the experiment and thus mask any centrally mediated effect at a later time. The absence of leakage to the periphery is supported by the fact that following injection of dye into the hypothalamus, the maximum spread through the brain tissue after 30 minutes is less than 0.2 mm from the injection site. This would also indicate that the injected drug remains in the individual nucleus of the hypothalamus, suggesting a highly localised site of action (See figure 38c). The hypotension and bradycardia observed appear to be centrally mediated since following peripheral administration, adrenaline caused an increase in blood pressure and a reflex bradycardia which lasts approximately 5 minutes. In addition, it is possible that the initial increase in blood pressure may not involve neuronal mechanisms because it is not dose dependant.

Following injection of adrenaline into the posterior

hypothalamus, an immediate hypotension and bradycardia were observed. Pretreatment with icv propranolol significantly reversed the hypotension and abolished the bradycardia, suggesting that these responses were a result of interaction at central beta- adrenoceptors.

Philippu et al (1971) reported that following superfusion of the hypothalamus of anaesthetised cats with adrenaline, a dose dependant rise in blood pressure was recorded which was significantly reduced following spinal transection. This superfusion of adrenaline also enhanced the release of radioactive amines from the hypothalamus suggesting that the rise in blood pressure, at least in part, was a result of an action within the hypothalamus.

3.4.5.3. Injection of beta- adrenoceptor agonists into the hypothalamus and pretreatment with beta- adrenoceptor blockers.

Injection of isoprenaline into both the anterior and posterior hypothalamus caused hypotension and tachycardia (see figures 33 and 34). The duration of hypotension was significantly decreased by pretreatment with propranolol icv, but the maximum degree of hypotension was unaffected. However, chronic treatment with oral propranolol reduced the hypotension produced by isoprenaline at both injection sites. It is likely that following chronic oral dosing

with propranolol, there will be significant amounts concentrated in the hypothalamus. Myers et al (1975) demonstrated a hypothalamus-to-plasma ratio of 15:1 following a single iv injection of propranolol in rabbits.

These results suggest that the presence of propranolol in the hypothalamus as a result of chronic oral dosing is much more effective at blocking the centrally mediated responses to isoprenaline than a single central injection of propranolol. This could be a result of insufficient propranolol being present in the hypothalamus following icv injection, although the response to adrenaline was attenuated by icv propranolol (see 3.4.5.2.).

Pretreatment with icv atenolol significantly reduced the duration of hypotension produced by isoprenaline injected into the posterior hypothalamus, whereas the tachycardia was significantly potentiated (see figures 35a and 35b). Pretreatment with icv ICI 118,551 significantly potentiated the hypotension at 1 and 2 minutes following injection of isoprenaline, but this soon returned to similar values as the untreated group. The tachycardia was potentiated by ICI 118,551 (see figures 36a and 36b).

Superfusion of the posterior hypothalamus of anaesthetised cats with isoprenaline has been shown to enhance the pressor response to electrical stimulation of the

hypothalamus (Philippu and Kittel, 1977; Philippu and Stroehl, 1978). This suggests that beta- adrenoceptors are present in the posterior hypothalamus and are involved in the increase in blood pressure elicited by hypothalamic stimulation.

Isoprenaline has also been reported to facilitate noradrenaline and dopamine release in the rat hypothalamus, thought to be a result of stimulation of pre-synaptic beta-adrenoceptors (Ueda et al, 1983). This was also demonstrated by Dietl et al (1981) in the cat hypothalamus. The facilitatory effects were abolished by pretreatment with beta$_1$- and beta$_2$- antagonists, suggesting the co-existence of pre-synaptic beta$_1$- and beta$_2$-adrenoceptors in the hypothalamus. Alternatively, there is the possibility that these beta- adrenoceptors do not exhibit the same selectivity as peripheral beta-adrenoceptors.

Thus, the majority of information concerning isoprenaline in the hypothalamus has examined its effect upon responses to electrical stimulation and is thought to involve an interaction with pre-synaptic beta- adrenoceptors. In this study, I have examined the effect of an injection of isoprenaline into the hypothalamus and pretreatment with beta- adrenoceptor antagonists. It appears that atenolol blocks the hypotension produced by isoprenaline to a

greater degree than ICI 118,551, suggesting that the hypotension produced by isoprenaline may be mediated via beta$_1$- adrenoceptors in the hypothalamus.

As a result of the low lipophilicity of atenolol, it is possible that only low levels are present in the hypothalamus following icv injection and this may explain why the attenuation of the hypotension to isoprenaline is not complete. The possibility of the isoprenaline induced hypotension being mediated via beta$_1$- adrenoceptors is also supported by the fact that blockade of beta$_2$- adrenoceptors by ICI 118,551 resulted in a potentiation of hypotension.

However, hypotension and tachycardia are caused by injection of the beta$_2$- adrenoceptor agonist, clenbuterol (see figures 31 and 32). Pretreatment with propranolol more readily antagonises the hypotension produced by clenbuterol when injected into the posterior hypothalamus, indicating that the involvement of beta$_2$- adrenoceptors cannot be ruled out completely.

Since pretreatment with icv propranolol potentiates the tachycardia caused by isoprenaline, it is possible that the attenuation of hypotension could just be result of this enhanced tachycardia. In order to eliminate this possibility, injections into animals pretreated with atenolol were made. It was hoped that pretreatment with

atenolol would eliminate any increase in heart rate because the cardiac beta1- adrenoceptors would have been blocked by the drug. It also had the advantage that icv injection of propranolol had no effect on blood pressure and heart rate in these pretreated animals and so both groups are at the same baseline values at the start of injection of isoprenaline.

Injection of isoprenaline into the anterior hypothalamus of animals pretreated with atenolol produced a fall in blood pressure and a small increase in heart rate (see figures 37a and 37b). The degree of hypotension was greater in these animals pretreated with atenolol than had been observed in untreated rats. Pretreatment with icv propranolol significantly reduced this hypotension, showing that the reduction in hypotension was not simply a reflection of enhanced tachycardia. It is unlikely that there would be a significant amount of atenolol in the brain following oral dosing since atenolol does not cross the blood brain barrier to any great extent by virtue of its low lipophilicity. This is also demonstrated by the fact that hypotension is still produced by isoprenaline injected into the hypothalamus of animals pretreated with atenolol, whereas pretreatment with oral propranolol significantly reversed the hypotension induced by intrahypothalamic isoprenaline.

Because of the uncertainty of how much beta- adrenoceptor antagonist would reach the hypothalamus following icv injection, it was decided to try an injection of propranolol and isoprenaline into the anterior hypothalamus. Injection of propranolol alone into the anterior hypothalamus caused a small increase in mean arterial pressure accompanied by a large bradycardia (see figures 38a and 38b). Lavy and Stern (1970) demonstrated decreases in heart rate following implantation of propranolol into the anterior hypothalamus, which they suggested was a result of a reduction in the activity of noradrenaline in the anterior hypothalamus causing a preponderance of the parasympathetic activity and hence a reduction in heart rate.

Injection of propranolol and isoprenaline simultaneously into the anterior hypothalamus resulted in biphasic changes in blood pressure and heart rate, which could not be explained simply by addition of the responses obtained to the two drugs injected singly. Thus, the responses to an injection of propranolol and isoprenaline together cannot be explained by simple competition between propranolol and isoprenaline for beta- adrenoceptors in the hypothalamus. The difficulty in interpreting the responses in terms of the effects of each drug precluded the use of this type of experiment. These problems could be overcome by the use of multibarrelled cannulae which would enable the injection of

more than one drug at separate times without the removal of the cannula between injections.

In conclusion, the results of injections into the hypothalamus of anaesthetised New Zealand rats implicate beta- adrenoceptors in the hypotensive and tachycardic responses to beta- adrenoceptor agonists. Adrenaline, which acts equally at both alpha- and beta- adrenoceptors, caused a biphasic change in arterial pressure when injected into the anterior hypothalamus and hypotension when injected into the posterior hypothalamus. Noradrenaline, which has a greater affinity for alpha- than beta- adrenoceptors caused a hypertension in the anterior and hypotension in the posterior hypothalamus.

From results obtained from injections into the anterior hypothalamus, it would appear that activation of alpha- adrenoceptors causes hypertension whereas hypotension is a result of an interaction with beta- adrenoceptors. In general, injections into the posterior hypothalamus are more likely to produce hypotensive responses than injections into the anterior hypothalamus.

It is obvious that responses to intrahypothalamic injections in conscious animals would be helpful in interpreting the central mechanisms involved. However, in this study, there was not time to develop a suitable method

to facilitate injections into the hypothalamus of conscious animals.

3.5. Injections into the cerebral ventricle of anaesthetised Japanese Okamoto spontaneously hypertensive and Wistar Kyoto normotensive rats.

3.5.1. Icv injection of isoprenaline in Wistar rats anaesthetised with Hypnorm/Hypnovel and pretreatment with propranolol.

Injection of 5 mcg isoprenaline into the left lateral cerebral ventricle of Wistar rats anaesthetised with Hypnorm/Hypnovel caused a fall in mean arterial pressure, reaching a maximum of 19 mmHg at 4 minutes (see figure 39a). The hypotension was not significantly reduced by 12 mcg propranolol iv, but was significantly reduced by 30 mcg propranolol icv. Following pretreatment with propranolol, tachycardia following isoprenaline was observed which was not present in untreated animals (see figure 39b).

3.5.2. Icv injection of isoprenaline into Japanese Okamoto spontaneously hypertensive and Wistar Kyoto normotensive rats. (2.2.2.)

In Japanese Okamoto spontaneously hypertensive rats anaesthetised with Hypnorm/Hypnovel no significant change in mean arterial pressure was seen following icv injection of isoprenaline (see figure 40a), but a tachycardia of 77 bpm was observed after 20 minutes (see figure 40b).

In both Wistar and Japanese Okamoto rats anaesthetised with Inactin, icv injection (1-5 mcg) caused a dose dependant hypotension and tachycardia (see figures 41, 42 and 43). The degree of hypotension at each dose of isoprenaline was greater in the hypertensive animals, however the degree of tachycardia was smaller. The maximum values for hypotension and tachycardia are shown below:

Dose isopren. (mcg)	Wistar		Jap. Okamoto		Figure
	Hypoten. (mmHg)	Tachycard. (bpm)	Hypoten. (mmHg)	Tachycard. (bpm)	
1.0	7	47	14	42	41
3.0	7	100	22	80	42
5.0	32	128	44	101	43

3.5.3. The effect of pretreatment with propranolol on responses to icv isoprenaline in rats anaesthetised with Inactin. (2.2.2. and 2.2.4.)

The hypotension and tachycardia induced by 5 mcg isoprenaline icv in Wistar rats were significantly attenuated by pretreatment with 30 mcg propranolol icv to

13 mmHg and 77 bpm (see figure 44a and 44b). Chronic oral dosing with propranolol for 14 days abolished the hypotension to isoprenaline and attenuated the tachycardia to a greater extent than 30 mcg propranolol icv (44 bpm).

The hypotension observed in Japanese Okamoto rats was blocked by pretreatment with 30 mcg propranolol icv ($p<0.01$) and 60 mg/Kg orally for 14 days ($p<0.001$). The tachycardia was attenuated by icv propranolol to 56 bpm and abolished by chronic oral propranolol (see figures 45a and 45b).

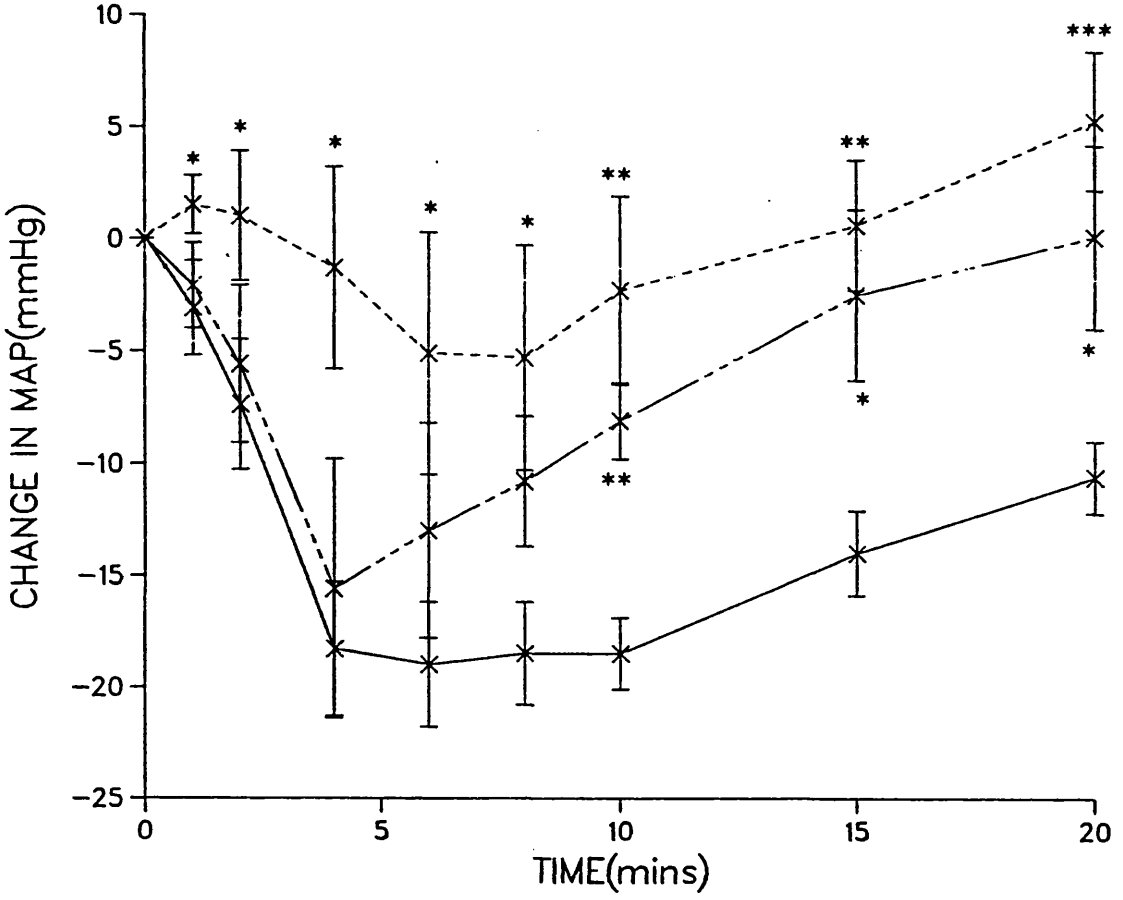

Figure 39a.

Figures 39a and 39b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in Wistar rats anaesthetised with Hypnorm/Hypnovel.

x———x No pretreatment (n=6) 62 mmHg, 460 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 89 mmHg, 400 bpm.

x—— - - ——x 12 mcg propranolol iv (n=6) 94 mmHg, 350 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

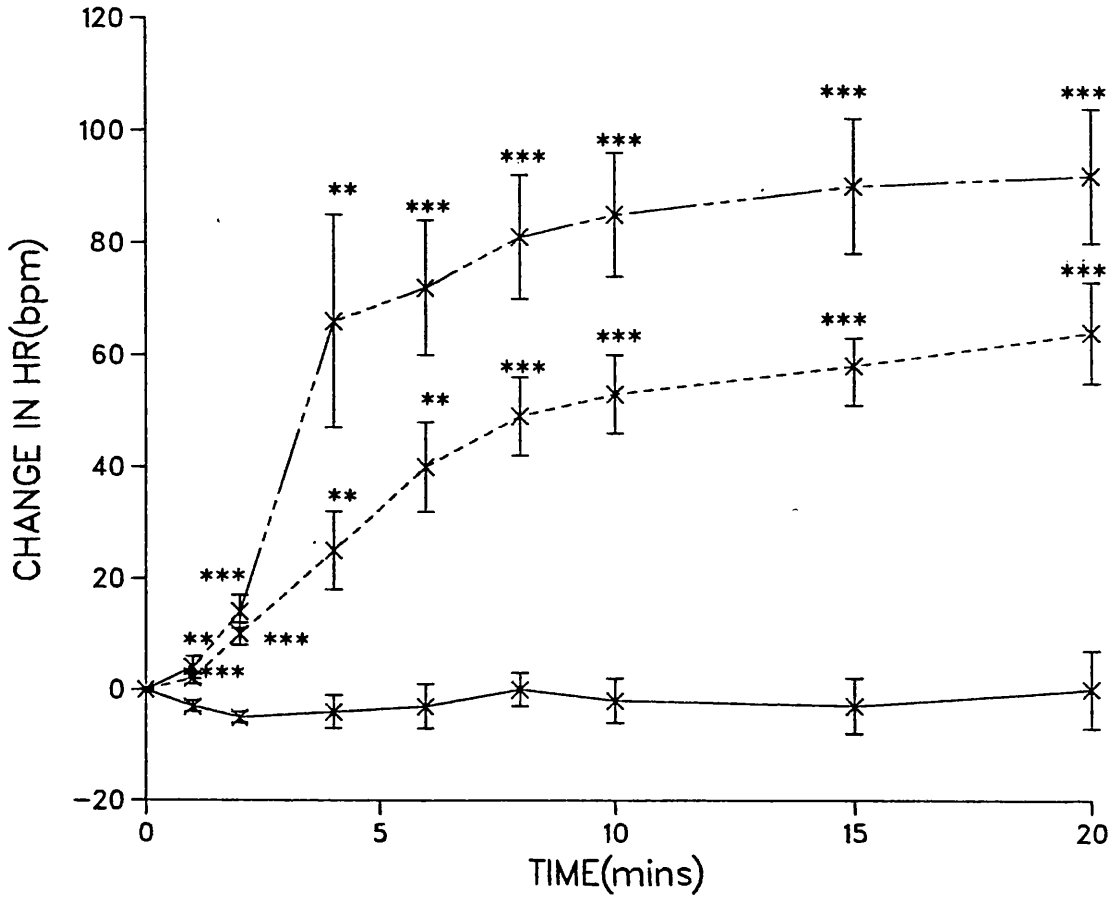

Figure 39b.

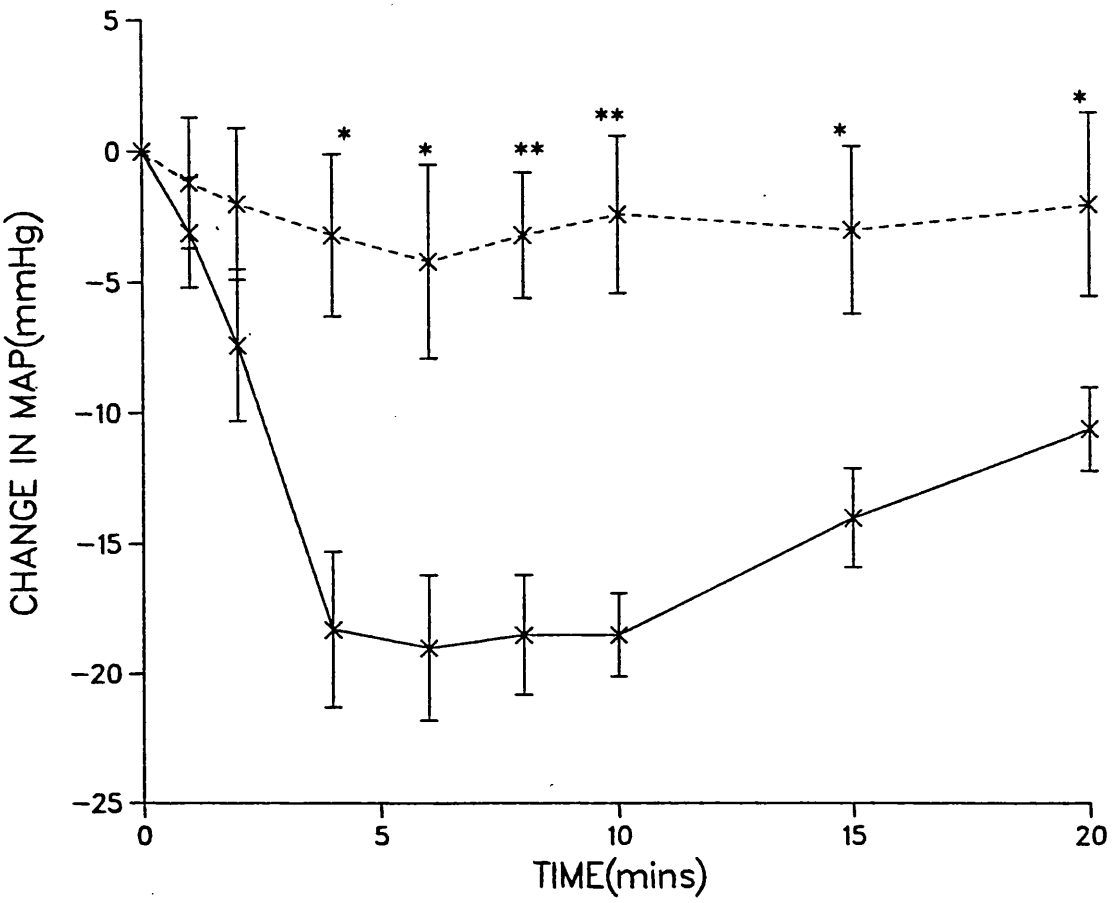

Figure 40a.

Figures 40a and 40b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in rats anaesthetised with Hypnorm/Hypnovel.

x————x Wistar rats (n=6) 62 mmHg, 460 bpm.

x- - - - - - -x Japanese Okamoto rats (n=6) 61 mmHg, 440 bpm.

Significant difference from Wistar rats denoted:

* $p<0.05$ ** $p<0.01$

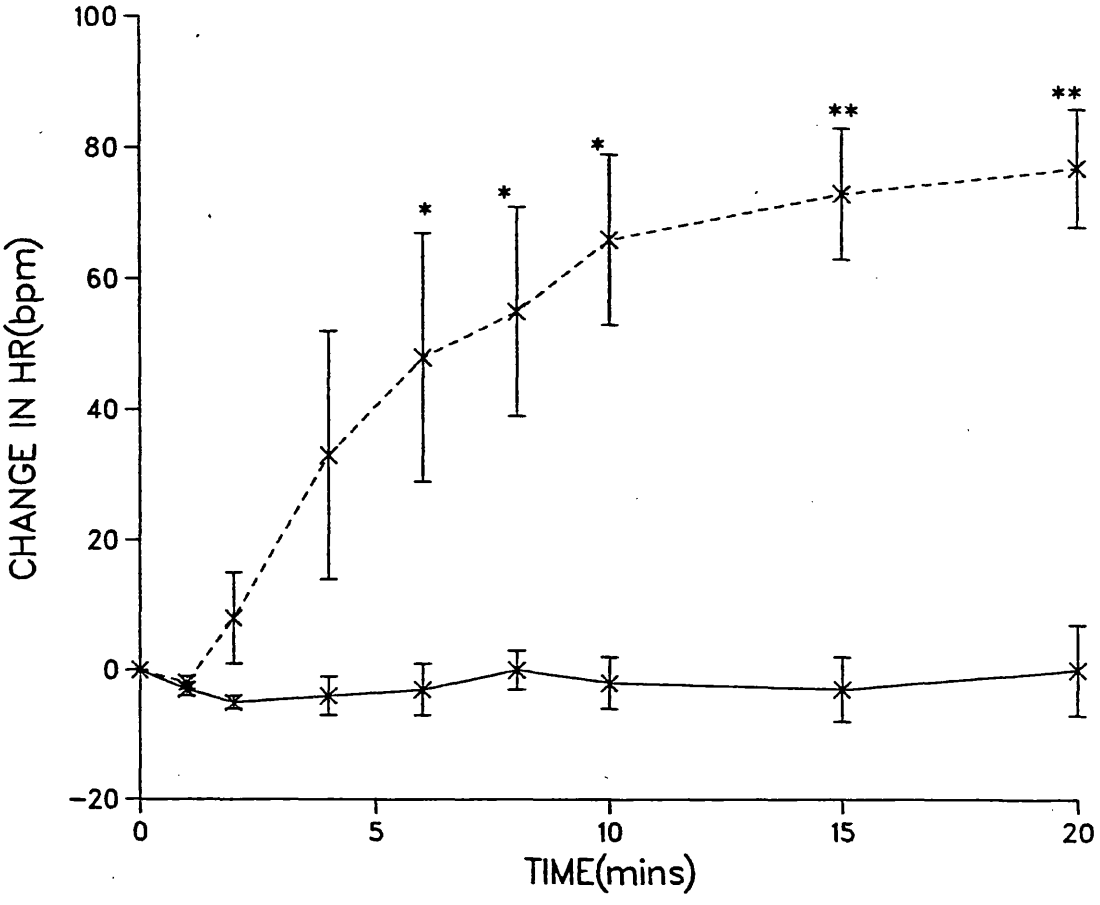

Figure 40b.

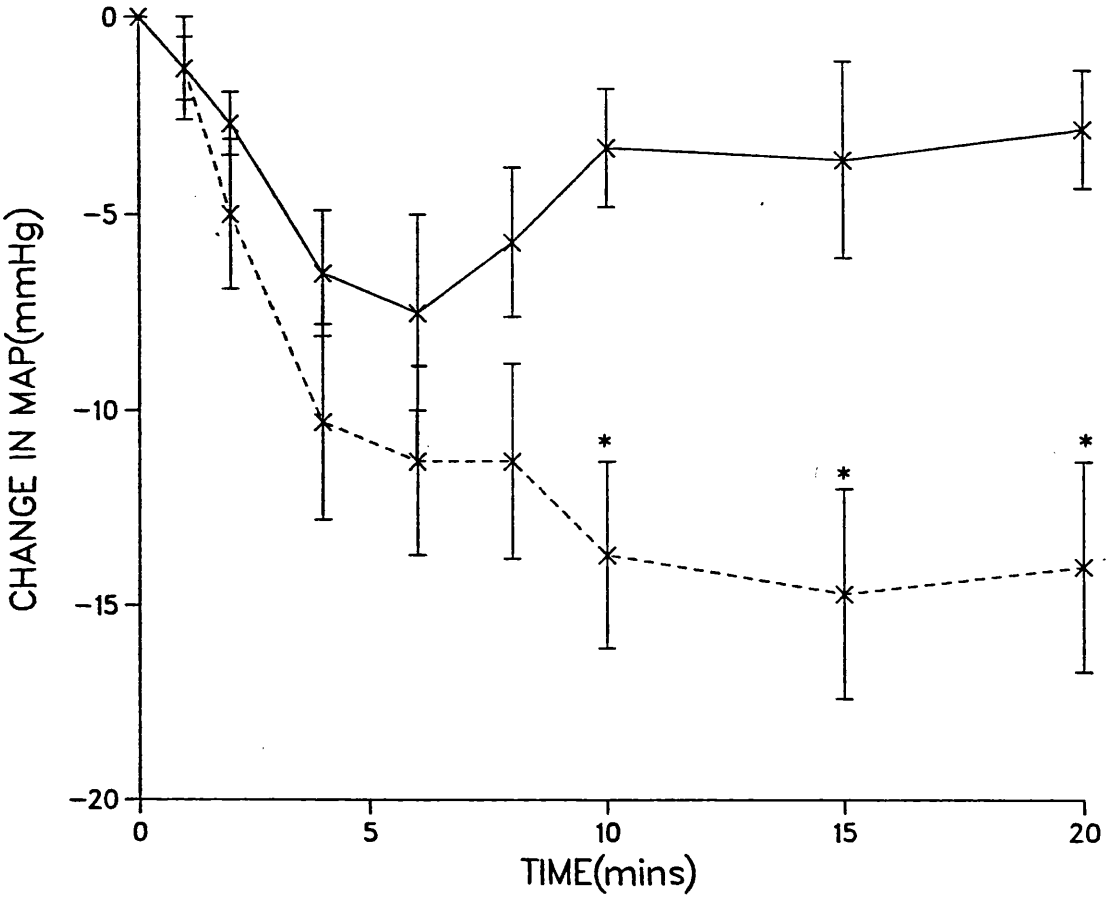

Figure 41a.

Figures 41a and 41b. Change in mean arterial pressure and heart rate following icv injection of 1 mcg isoprenaline in rats anaesthetised with Inactin.

x————x Wistar rats (n=6) 96 mmHg, 424 bpm.

x- - - - - -x Japanese Okamoto rats (n=6) 153 mmHg, 372 bpm.

Significant difference from Wistar rats denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

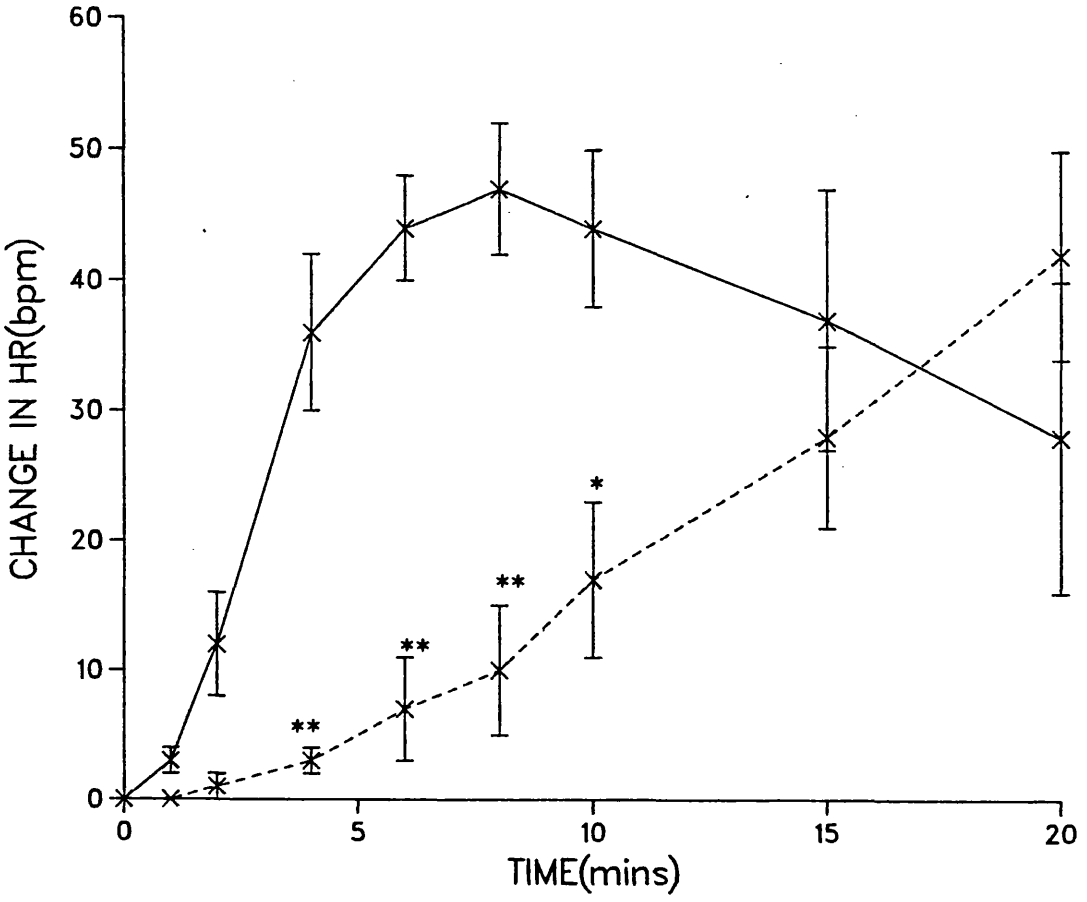

Figure 41b.

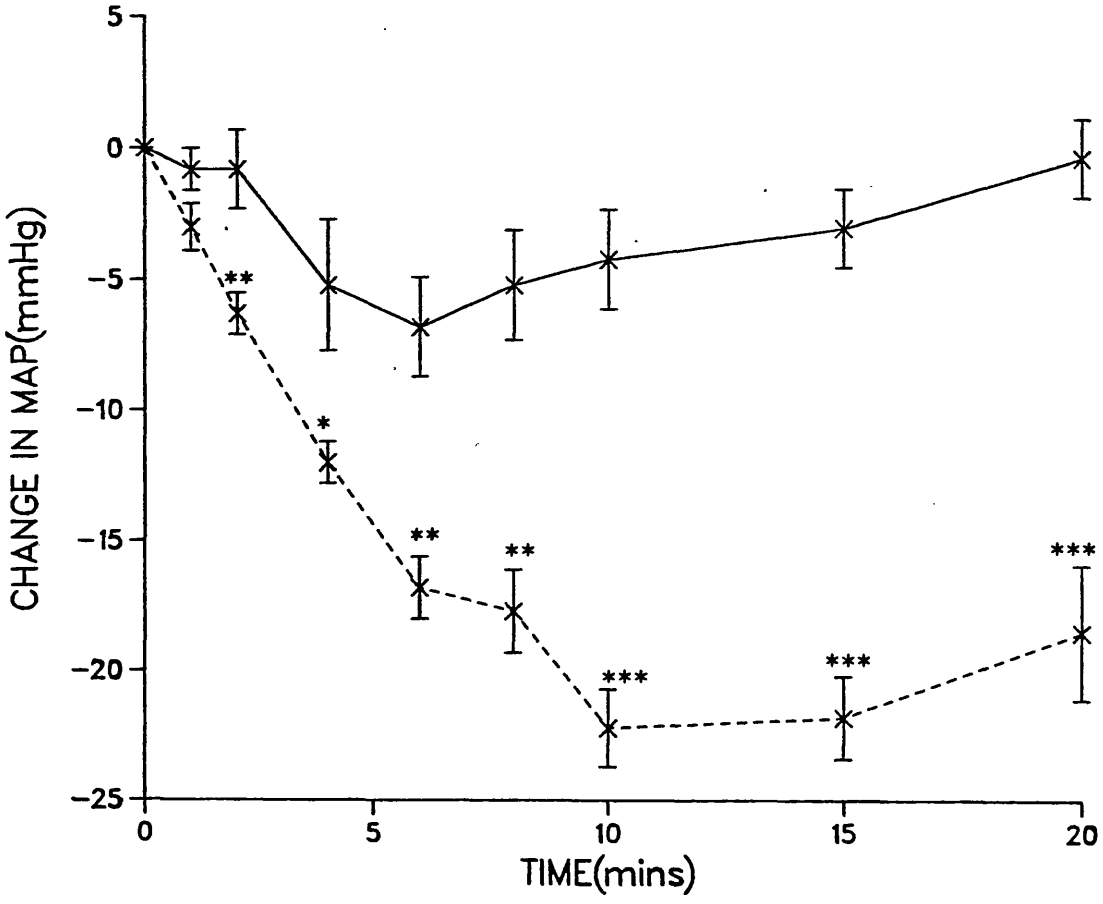

Figure 42a.

Figures 42a and 42b. Change in mean arterial pressure and heart rate following icv injection of 3 mcg isoprenaline in rats anaesthetised with Inactin.

x———x Wistar rats (n=6) 100 mmHg, 410 bpm.

x- - - - - -x Japanese Okamoto rats (n=6) 152 mmHg, 367 bpm.

Significant difference from Wistar rats denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

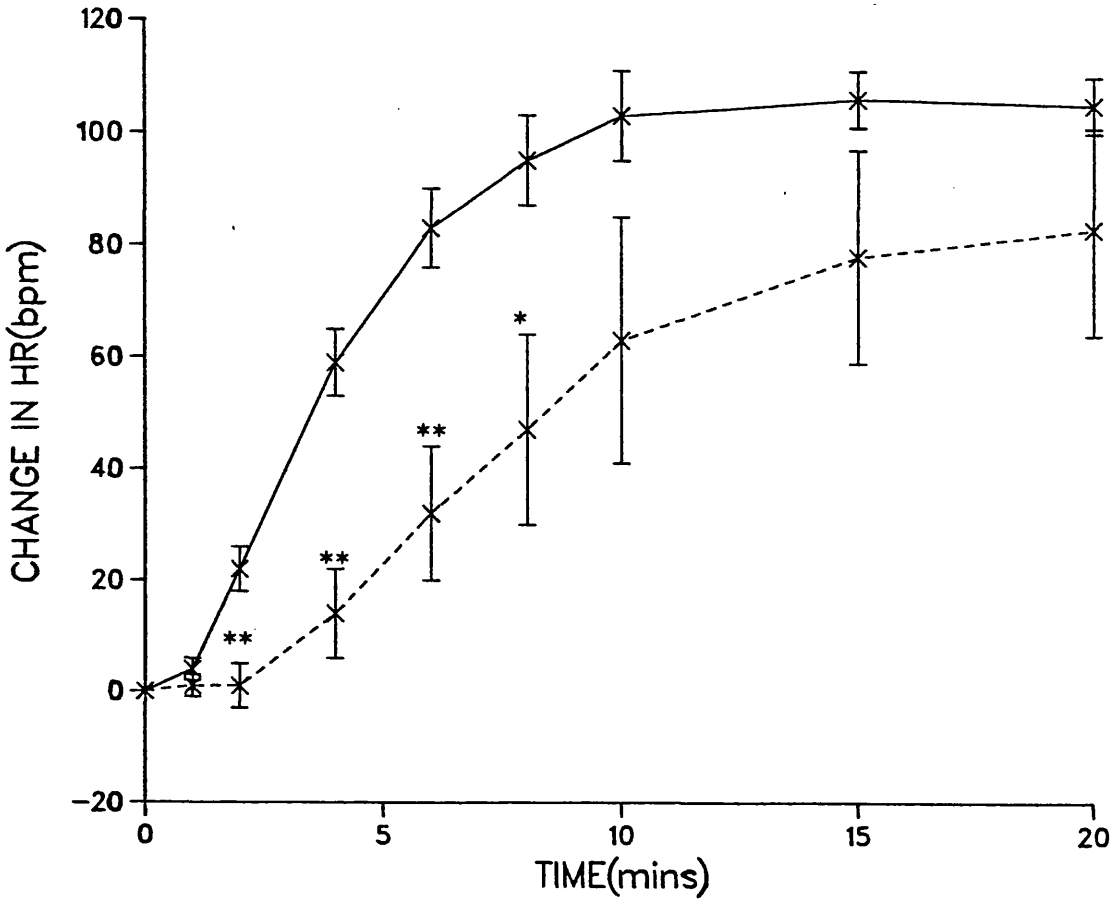

Figure 42b.

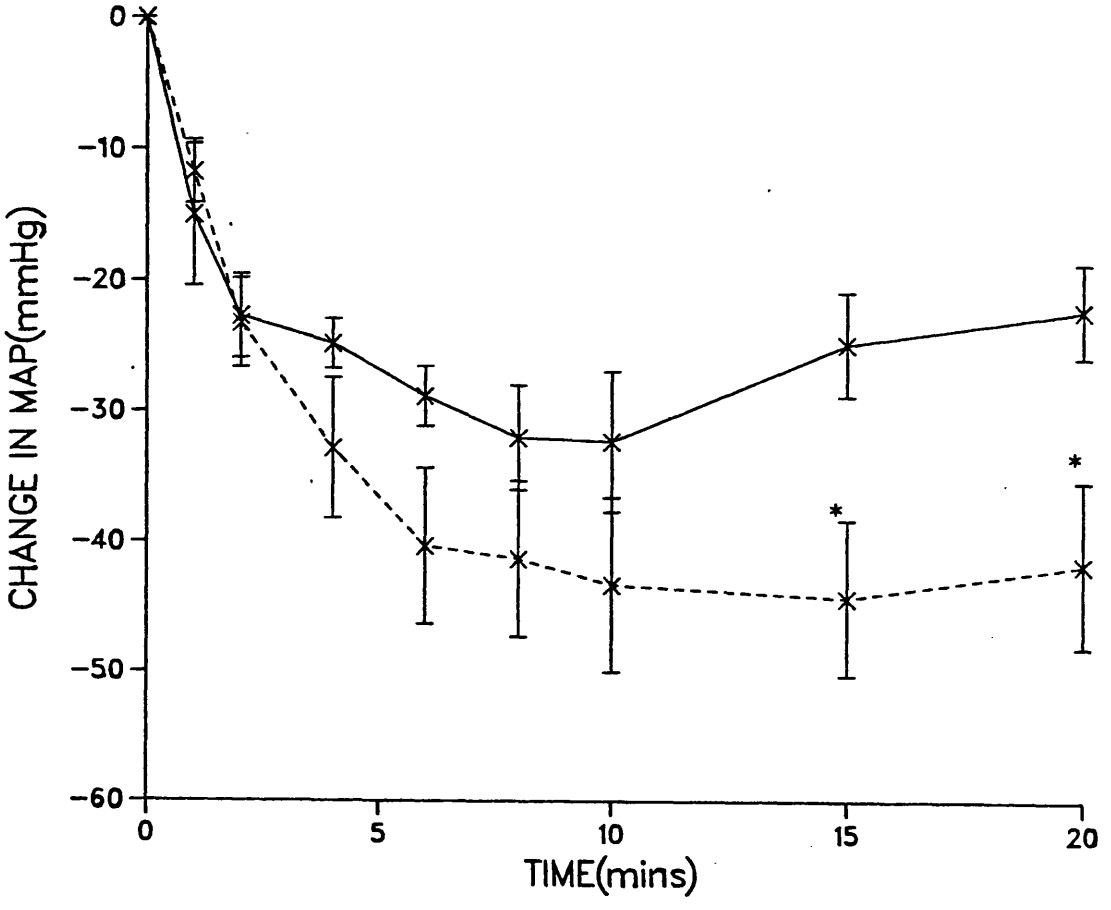

Figure 43a.

Figures 43a and 43b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in rats anaesthetised with Inactin.

x————x Wistar rats (n=6) 110 mmHg, 404 bpm.

x- - - - - - -x Japanese Okamoto rats (n=6) 149 mmHg, 375 bpm.

Significant difference from Wistar rats denoted:

* $p<0.05$

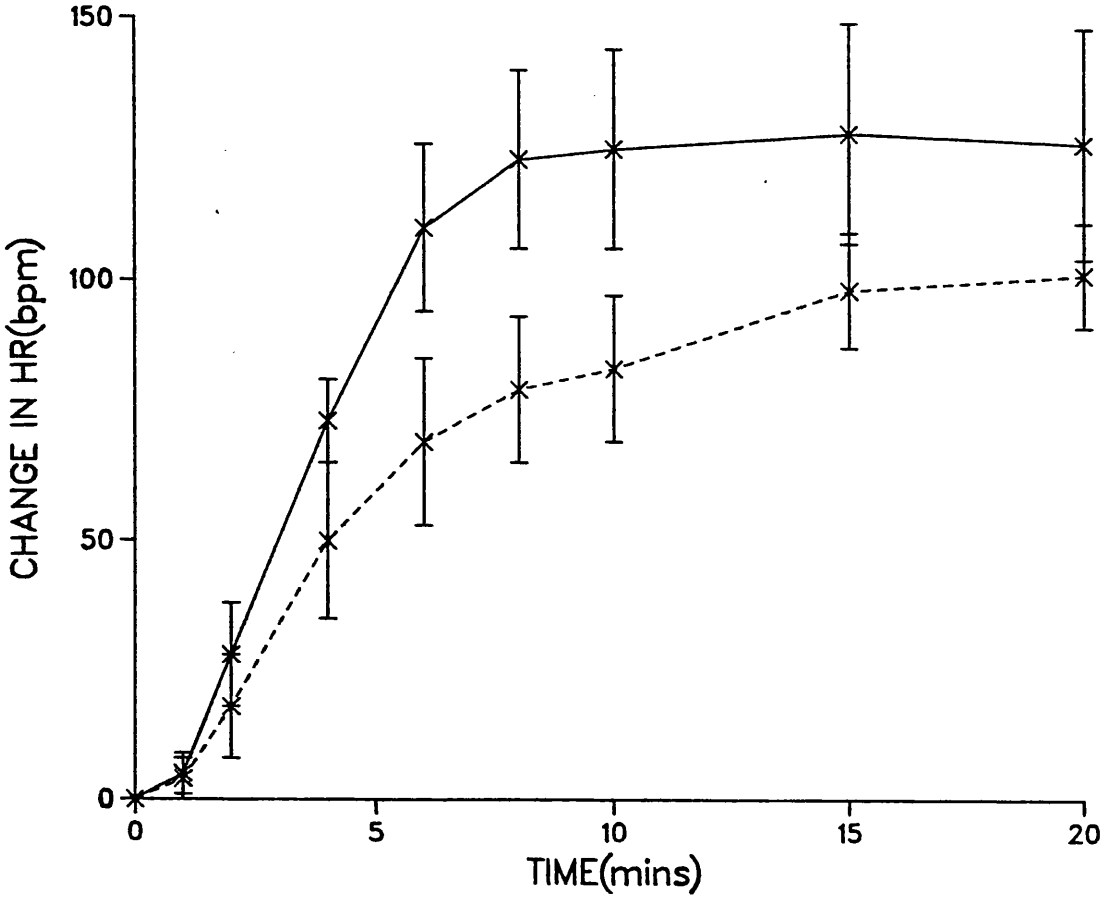

Figure 43b.

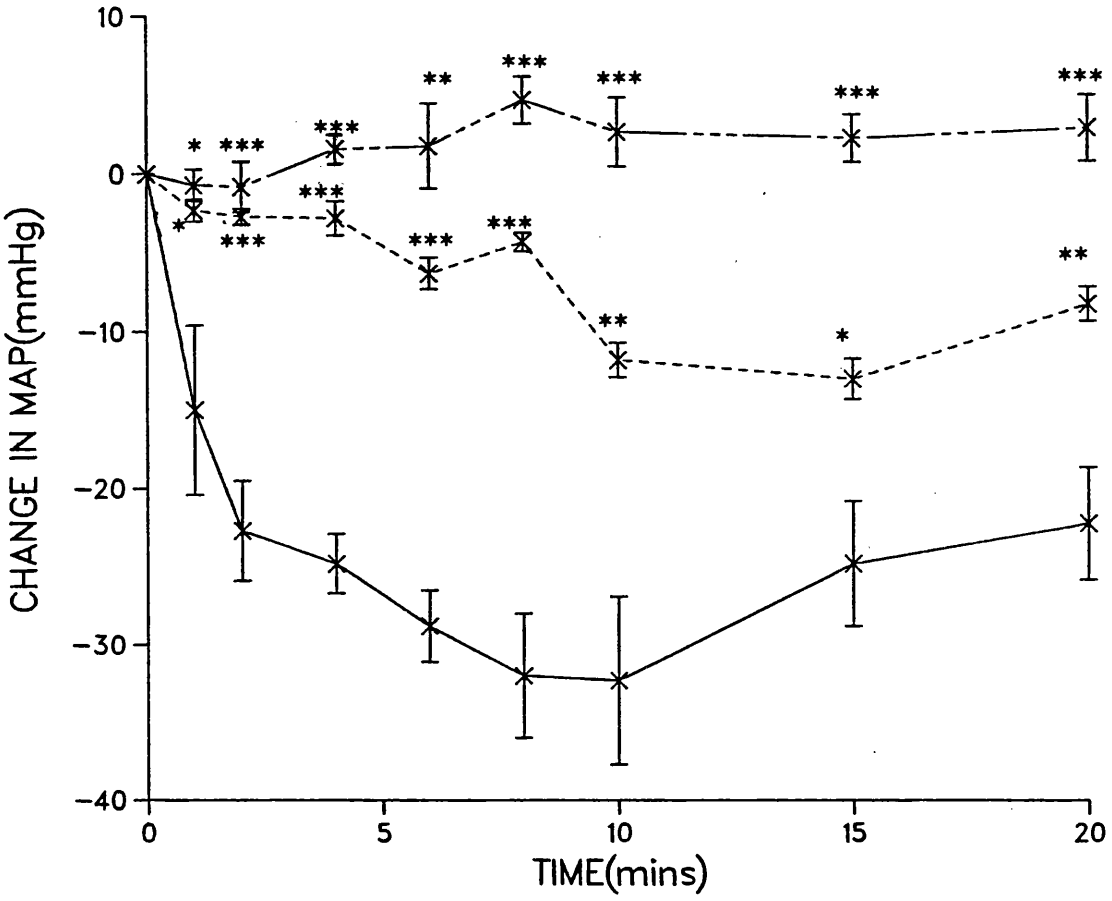

Figure 44a.

Figures 44a and 44b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in Wistar rats anaesthetised with Inactin.

x————x No pretreatment (n=6) 110 mmHg, 364 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=6) 70 mmHg, 380 bpm.

x—— - - - ——x 60 mg/Kg propranolol po daily for 14 days (n=6) 90 mmHg, 332 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

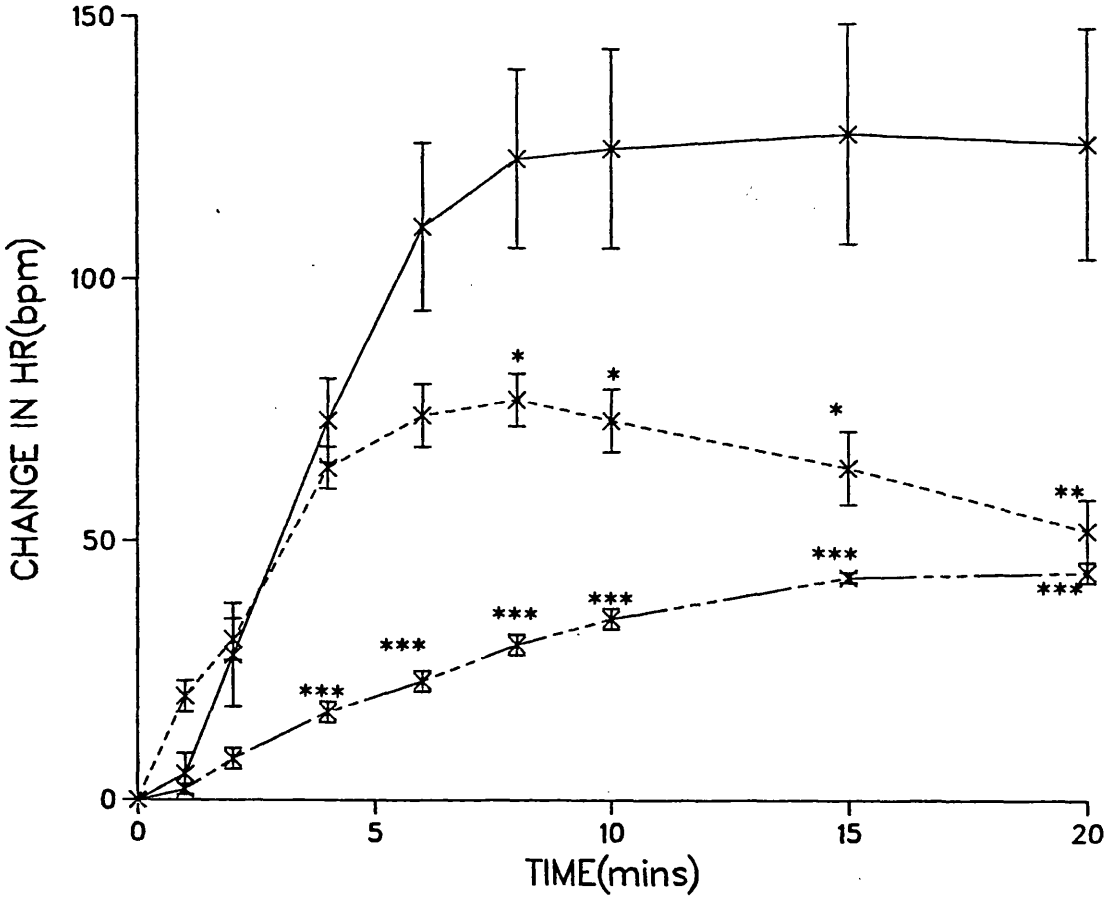

Figure 44b.

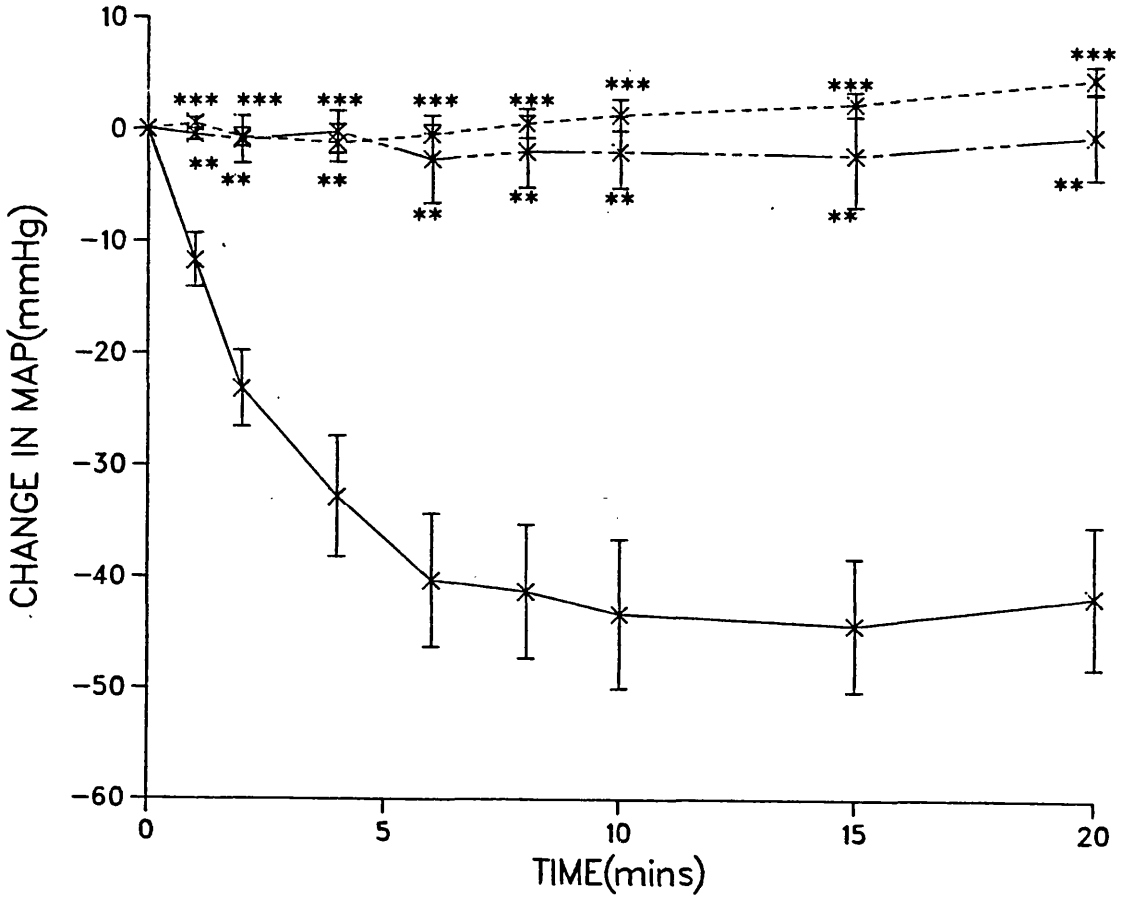

Figure 45a.

Figures 45a and 45b. Change in mean arterial pressure and heart rate following icv injection of 5 mcg isoprenaline in Japanese Okamoto rats anaesthetised with Inactin.

x————x No pretreatment (n=6) 149 mmHg, 375 bpm.

x- - - - - -x 30 mcg propranolol icv (n=6) 95 mmHg, 341 bpm.

x—— - - - -x 60 mg/Kg propranolol po daily for 14 days (n=6) 139 mmHg, 295 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

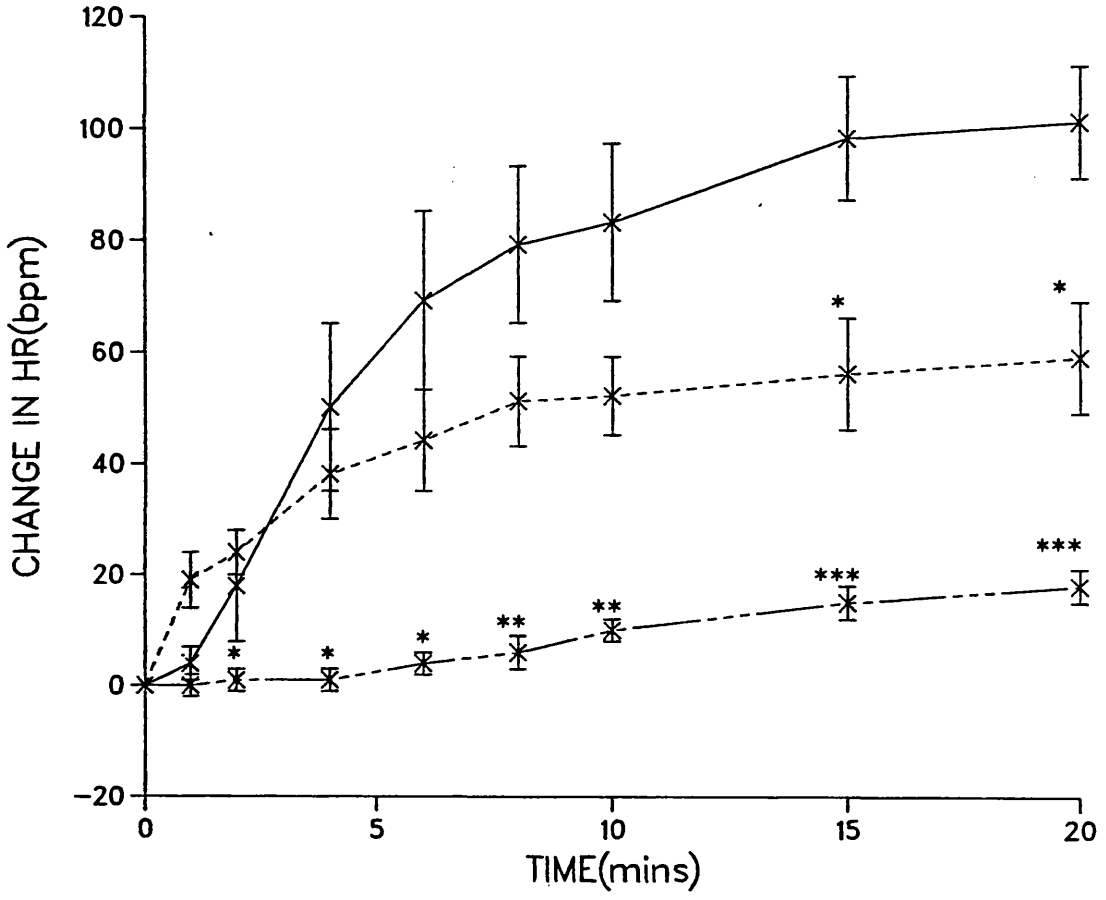

Figure 45b.

3.5.4. Discussion.

3.5.4.1. The influence of anaesthetics upon responses to centrally administered drugs.

Experiments using Wistar Kyoto normotensive and Japanese Okamoto spontaneously hypertensive rats illustrated the fact that not only is the presence of anaesthesia likely to alter the responses observed following central administration of drugs, but that the anaesthetic used may also make a lot of difference. Since this fact was most evident during this series of experiments, a more detailed discussion will be made.

The possibility that Hypnorm/Hypnovel may not be a suitable anaesthetic for use in Japanese Okamoto rats became apparent when no hypotension occurred following icv injection of isoprenaline into them. It was noted that the resting blood pressure and heart rate were approximately the same in both these and the Wistar Kyoto rats, 62 and 61 mmHg and 460 and 440 bpm respectively. From systolic blood pressure and heart rate measurements made using the tail cuff method (see 2.7 for details), it was known that the systolic blood pressure in the spontaneously hypertensive rat was around 220 mmHg whereas that of normotensive rats was around 150 mmHg. The heart rate for both groups was found to be approximately 450 bpm.

It is possible that Hypnorm/Hypnovel had reduced the arterial pressure in the spontaneously hypertensive rats to such an extent that further hypotension following icv injection of isoprenaline was not possible. The reason for this large degree of hypotension was thought to be the blockade of central or peripheral alpha- adrenoceptors by the fluanisone present in Hypnorm. Fluanisone is a neuroleptic drug of the butyrophenone class and is known to have an affinity resembling that of classic alpha-adrenoceptor agonists such as phentolamine and phenoxybenzamine (Peroutka et al, 1977). A relationship has been shown to exist between the clinical sedative and hypotensive effects of butyrophenone neuroleptics and their central alpha- adrenoceptor potency. It is possible that the hypertension present in Japanese Okamoto spontaneously hypertensive rats may in some way involve alpha-adrenoceptors and subsequent activity by fluanisone may result in the large hypotension seen in this study.

Anaesthesia with Inactin did not appear to affect the resting blood pressure of spontaneously hypertensive rats to as great an extent as Hypnorm/Hypnovel; the mean arterial pressure was approximately 150 mmHg as opposed to approximately 100 mmHg for Wistar rats. However, Inactin tended to produce a more pronounced bradycardia; the heart rate of spontaneously hypertensive rats was approximately 370 bpm, that of Wistar rats being 410 bpm. A reduction in

arterial pressure and heart rate has been reported following administration of Inactin (Cupples et al, 1982; Walker et al, 1983), with a decrement of renal blood flow being particularly apparent (Koeppen et al, 1979).

A particular problem associated with Inactin anaesthesia was that animals needed to be artificially respired during the experiment. This was thought to be a result of the animal being incapable of regulating its blood gasses. Buelke-Sam et al (1978) reported marked hypercapnia in animals anaesthetised with Inactin, leading to irregularities in heart rate and blood pressure.

Inactin has been shown to have a lesser effect upon central catecholamines than urethane (Chahl and Walker, 1981), although increases in central acetylcholine have been reported following anaesthesia with Inactin (Weigel et al,1978).

It was decided to use Inactin as the anaesthetic in subsequent experiments using Wistar and Japanese Okamoto rats as, although blood pressure and heart rate may still be affected by this anaesthetic, it seemed to be less detrimental than the Hypnorm/Hypnovel combination in the spontaneously hypertensive rat.

3.5.4.2. Icv injection in rats anaesthetised with Hypnorm/Hypnovel.

Injection of 5 mcg isoprenaline into the cerebral ventricle caused a fall in mean arterial pressure in Wistar rats which was comparable to that seen in New Zealand rats, but in Wistar rats no accompanying tachycardia was observed. Conversely, in Japanese Okamoto rats, tachycardia without hypotension occurred following icv isoprenaline. Thus, there appears to be a difference in responses between strains using the same anaesthetic.

The hypotension in Wistar rats was significantly reduced by pretreatment with icv propranolol (see figure 39a), suggesting this was a result of activation of beta-adrenoceptors by isoprenaline. Intravenous propranolol did not significantly alter the degree of hypotension, indicating that, at least in part, a centrally mediated mechanism is responsible for the response.

Tachycardia was observed following icv injection of isoprenaline in Wistar rats pretreated with propranolol either iv or icv (see figure 39b). It is possible that the bradycardia produced by propranolol facilitates the subsequent tachycardic response to icv isoprenaline, since no change in heart rate was observed in untreated animals.

3.5.4.3. Icv injections in rats anaesthetised with Inactin.

Following icv injection of isoprenaline in Wistar and Japanese Okamoto rats, hypotension and tachycardia were observed (see figures 41, 42 and 43). In general, the degree of hypotension was greater and tachycardia was less in Japanese Okamoto rats. In Wistar rats, the hypotension was significantly reduced by pretreatment with icv propranolol and abolished by chronic oral dosing with propranolol. The tachycardia was significantly reduced by both types of pretreatment (see figures 44a and 44b). The hypotension produced by 5 mcg isoprenaline icv in Japanese Okamoto rats was abolished by pretreatment with propranolol given either as a single icv injection or chronic oral doses (see figure 45a). Both pretreatments reduced the degree of tachycardia (see figure 45b).

The degree of hypotension in Wistar rats following 5 mcg isoprenaline icv was found to be 50% greater in animals anaesthetised with Inactin. Regardless of the anaesthetic used, pretreatment with icv propranolol in Wistar rats more readily attenuates the hypotension produced by icv isoprenaline than that observed in New Zealand rats. Thus, if a propranolol insensitive mechanism is involved as has been reported (Peres-Polon and Correa, 1984) it is not as apparent in Wistar rats as in New Zealand rats (see

3.2.11.4). In Japanese Okamoto rats, the hypotension is completely abolished by pretreatment with propranolol. This would agree more closely with studies in the cat (Gagnon and Melville, 1967) and the dog (Bhargava et al, 1972) where propranolol was found to block the response to central isoprenaline.

Thus, this series of experiments highlights the fact that the responses produced by icv injection of isoprenaline do not only rely upon the presence of anaesthesia, but also on the strain of animal and choice of anaesthetic.

3.6. Injection into the hypothalamus of anaesthetised Japanese Okamoto spontaneously hypertensive and Wistar Kyoto normotensive rats.

In this section of experiments, all animals were anaesthetised with Inactin.

3.6.1. Injection of isoprenaline into the hypothalamus of anaesthetised Wistar rats and pretreatment with propranolol. (2.2.5. and 2.2.6.)

Following injection of 5 mcg isoprenaline into the anterior or posterior hypothalamus, hypotension and tachycardia were observed. The magnitude of these responses was similar for each injection site, being 13 mmHg and 112 bpm, and 15 mmHg and 109 bpm for the anterior and posterior hypothalamus respectively (see figures 46 and 47). Pretreatment with propranolol (30 mcg icv) abolished the hypotension and reduced the tachycardia produced by isoprenaline injected into the anterior and posterior hypothalamus. Chronic oral dosing with propranolol (60 mg/Kg daily for 14 days) further reduced the degree of tachycardia and significantly reduced hypotension in both cases.

3.6.2. Injection of isoprenaline into the hypothalamus of anaesthetised Japanese Okamoto rats and pretreatment with propranolol. (2.2.5. and 2.2.6.)

Injection of isoprenaline into the anterior hypothalamus caused a fall in mean arterial pressure of 34 mmHg and an increase in heart rate of 56 bpm (see figures 48a and 48b). The hypotension was significantly ($p<0.05$) reduced to 10 mmHg by icv propranolol and abolished by oral dosing with propranolol for 14 days. Tachycardia was potentiated to 125 bpm by pretreatment with icv propranolol but abolished by chronic oral dosing with propranolol.

Injection of isoprenaline into the posterior hypothalamus caused a fall in mean arterial pressure of 18 mmHg and an increase in heart rate of 92 bpm. The degree of hypotension was unaffected by pretreatment with icv propranolol, although the duration of hypotension was attenuated. Tachycardia was potentiated by this pretreatment. Chronic oral dosing with propranolol abolished both responses to isoprenaline (see figures 49a and 49b).

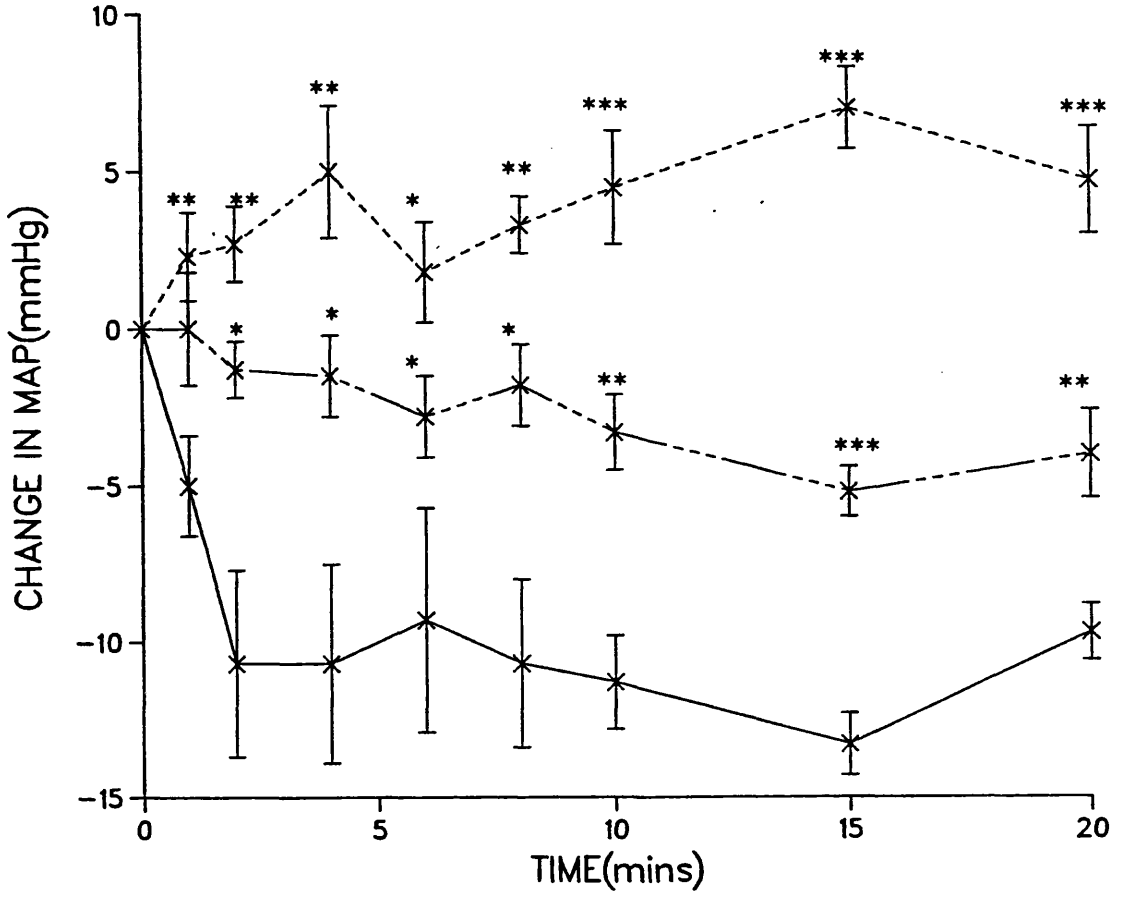

Figure 46a.

Figures 46a and 46b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the anterior hypothalamus of Wistar rats.

x————x No pretreatment (n=6) 75 mmHg, 400 bpm.

x- - - - -x 30 mcg propranolol icv (n=6) 90 mmHg, 398 bpm.

x——- - - -x 60 mg/Kg propranolol po daily for 14 days (n=6) 89 mmHg, 300 bpm.

Significant difference from no pretreatment group denoted:

* p<0.05 ** p<0.01 *** p<0.001

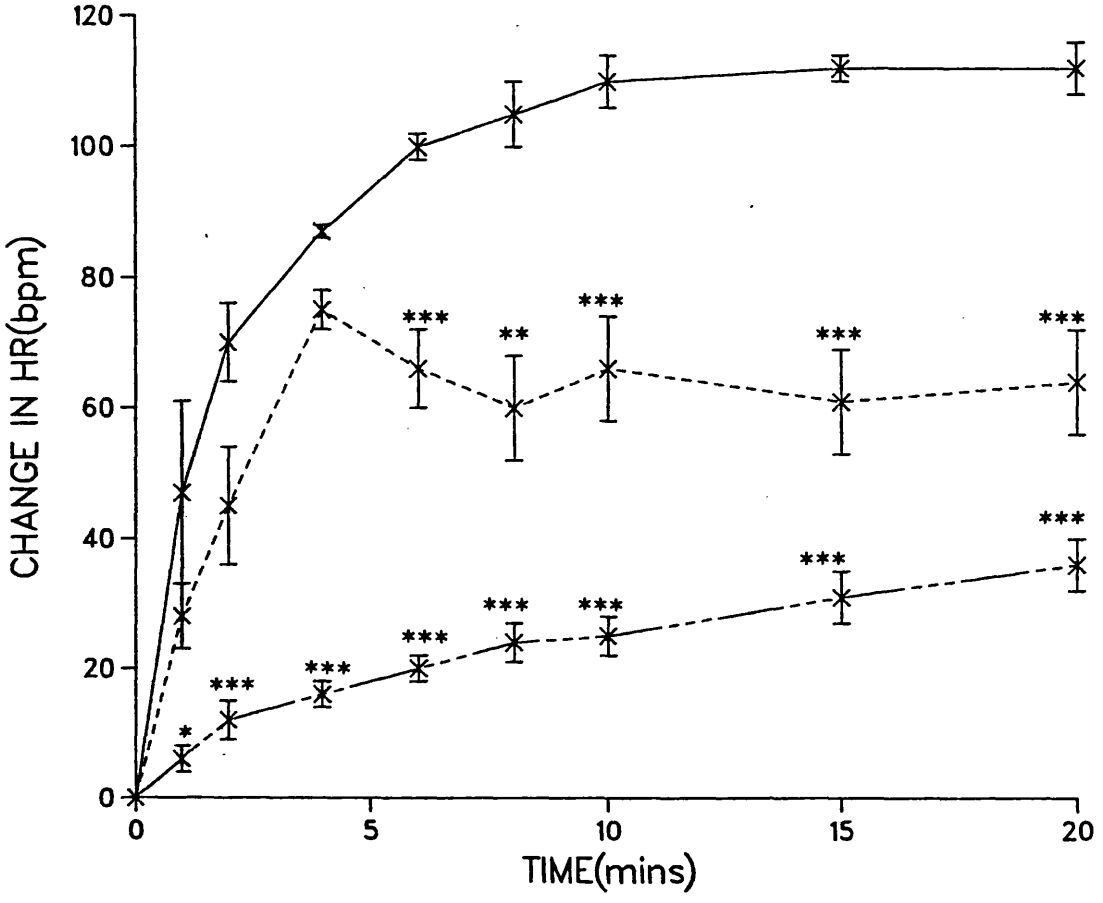

Figure 46b.

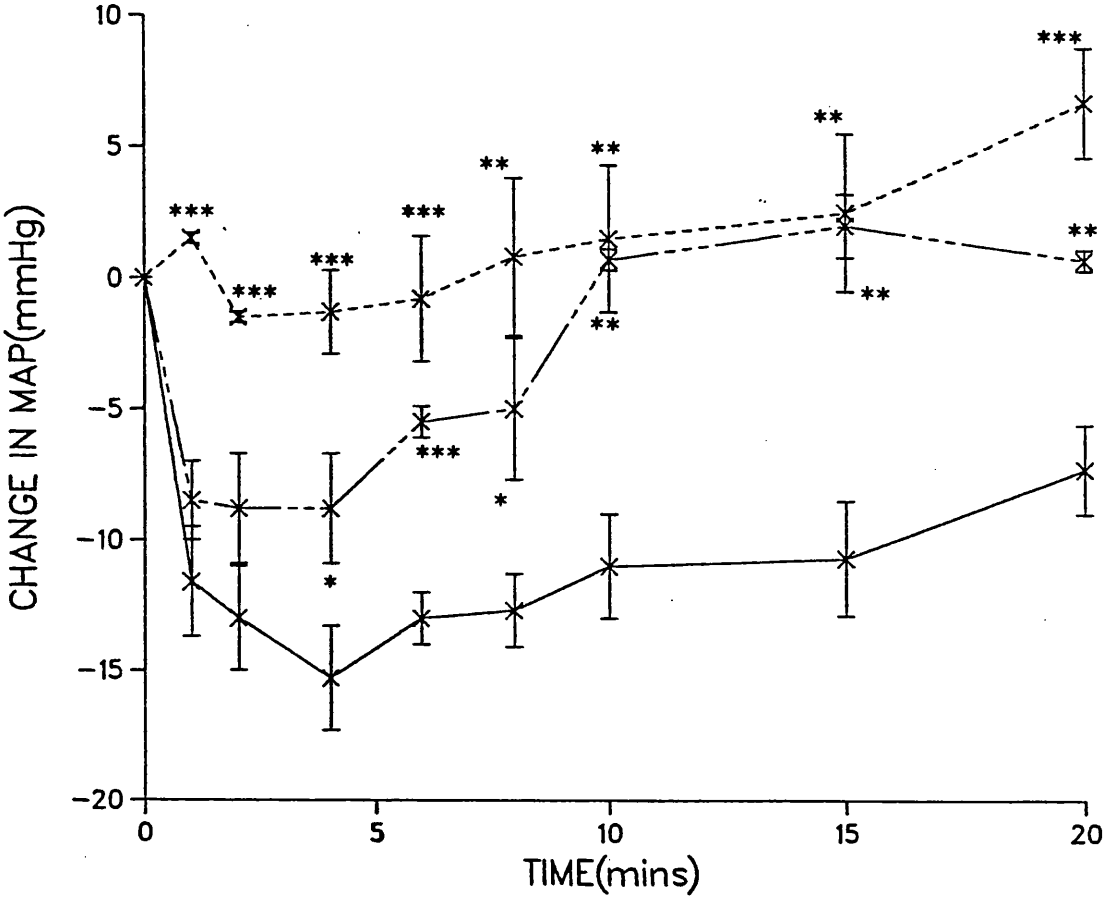

Figure 47a.

Figures 47a and 47b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the posterior hypothalamus of Wistar rats.

x————x No pretreatment (n=6) 67 mmHg, 377 bpm.

x- - - - -x 30 mcg propranolol icv (n=6) 78 mmHg, 400 bpm.

x——-——x 60 mg/Kg propranolol po daily for 14 days (n=6) 124 mmHg, 350 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

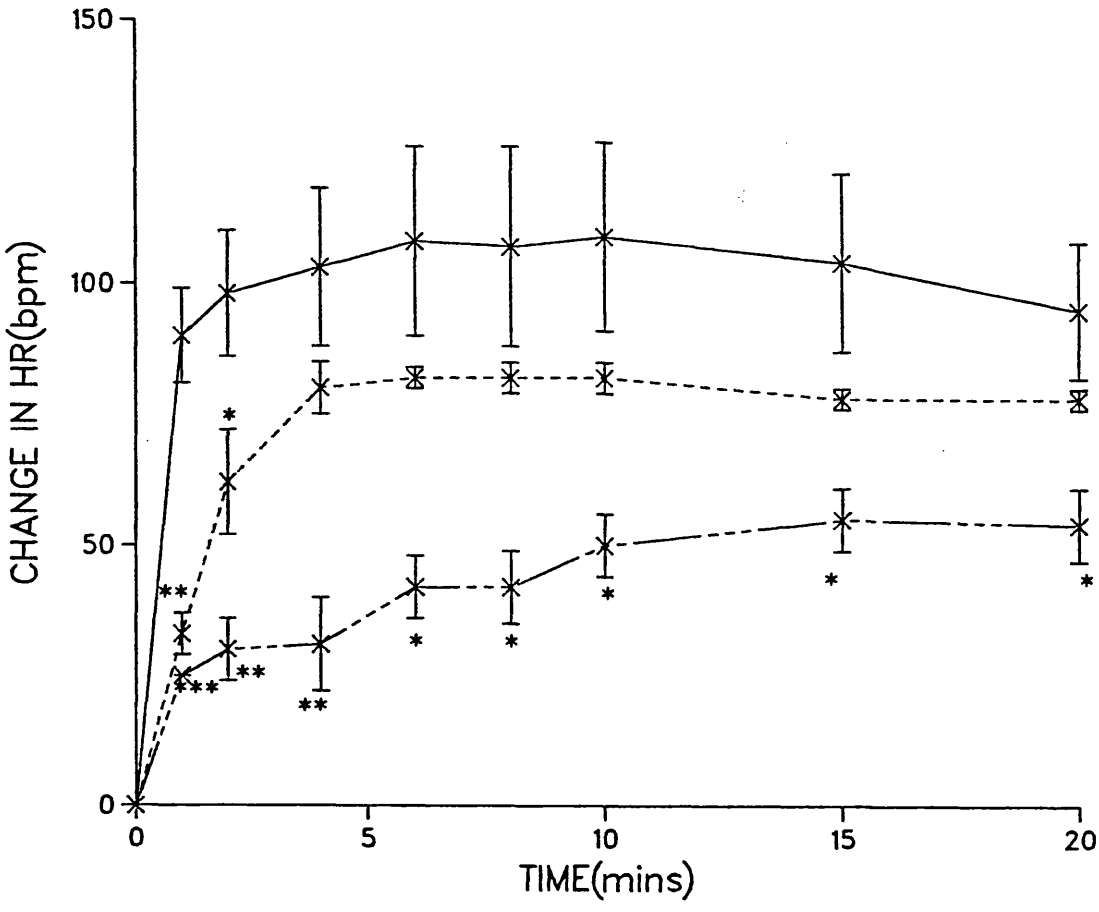

Figure 47b.

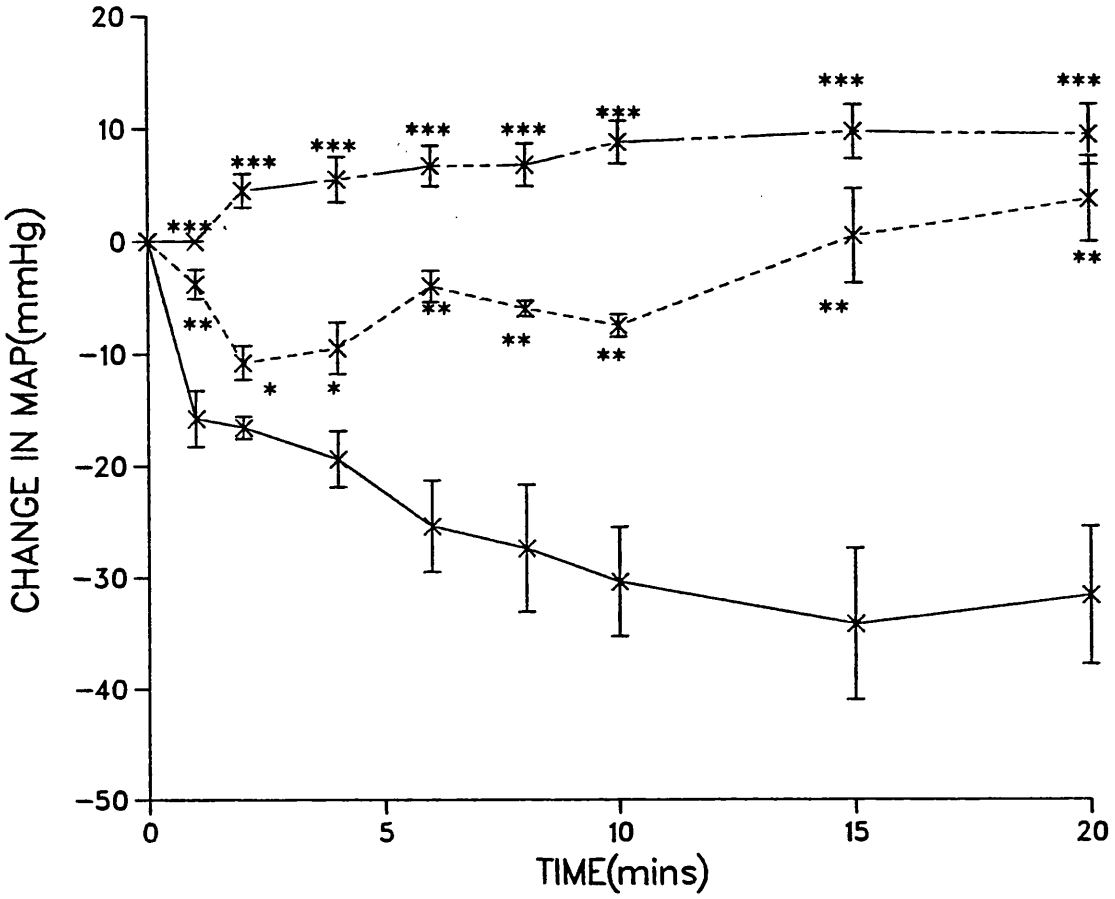

Figure 48a.

Figures 48a and 48b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the anterior hypothalamus of Japanese Okamoto rats.

x————x No pretreatment (n=6) 140 mmHg, 372 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=6) 132 mmHg, 360 bpm.

x——·-·——x 60 mg/Kg propranolol po daily for 14 days (n=6) 118 mmHg, 305 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

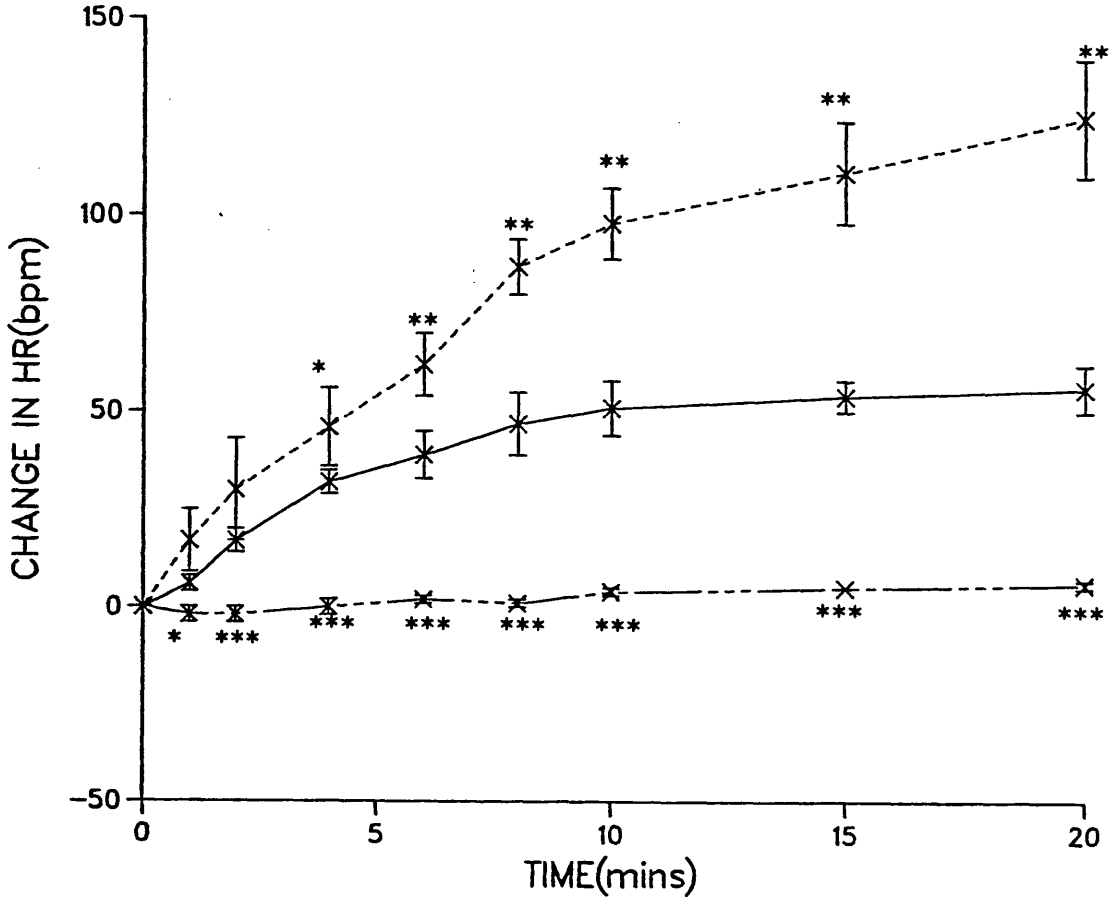

Figure 48b.

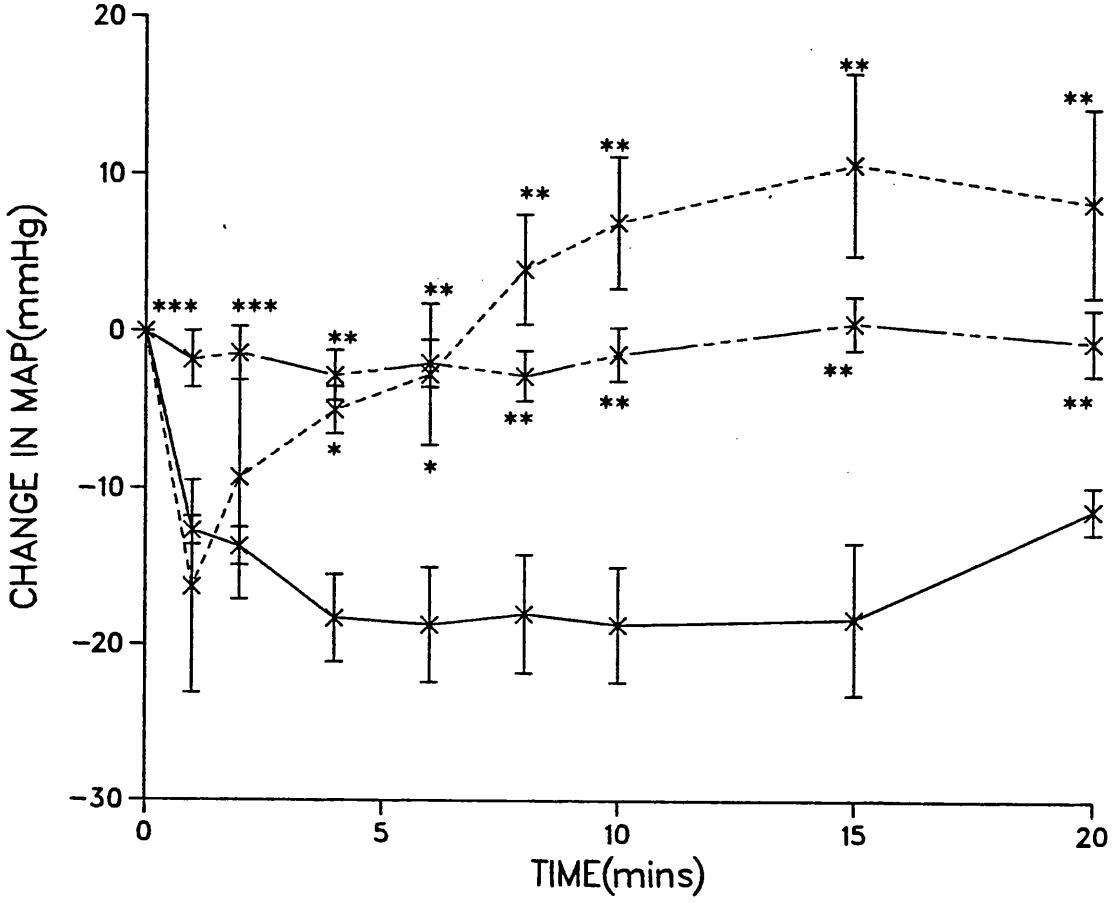

Figure 49a.

Figures 49a and 49b. Change in mean arterial pressure and heart rate following injection of 5 mcg isoprenaline into the posterior hypothalamus of Japanese Okamoto rats.

x————x No pretreatment (n=6) 138 mmHg, 418 bpm.

x- - - - - - -x 30 mcg propranolol icv (n=6) 130 mmHg, 375 bpm.

x—— ·-·-·——x 60 mg/Kg propranolol po daily for 14 days (n=6) 123 mmHg, 297 bpm.

Significant difference from no pretreatment group denoted:

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

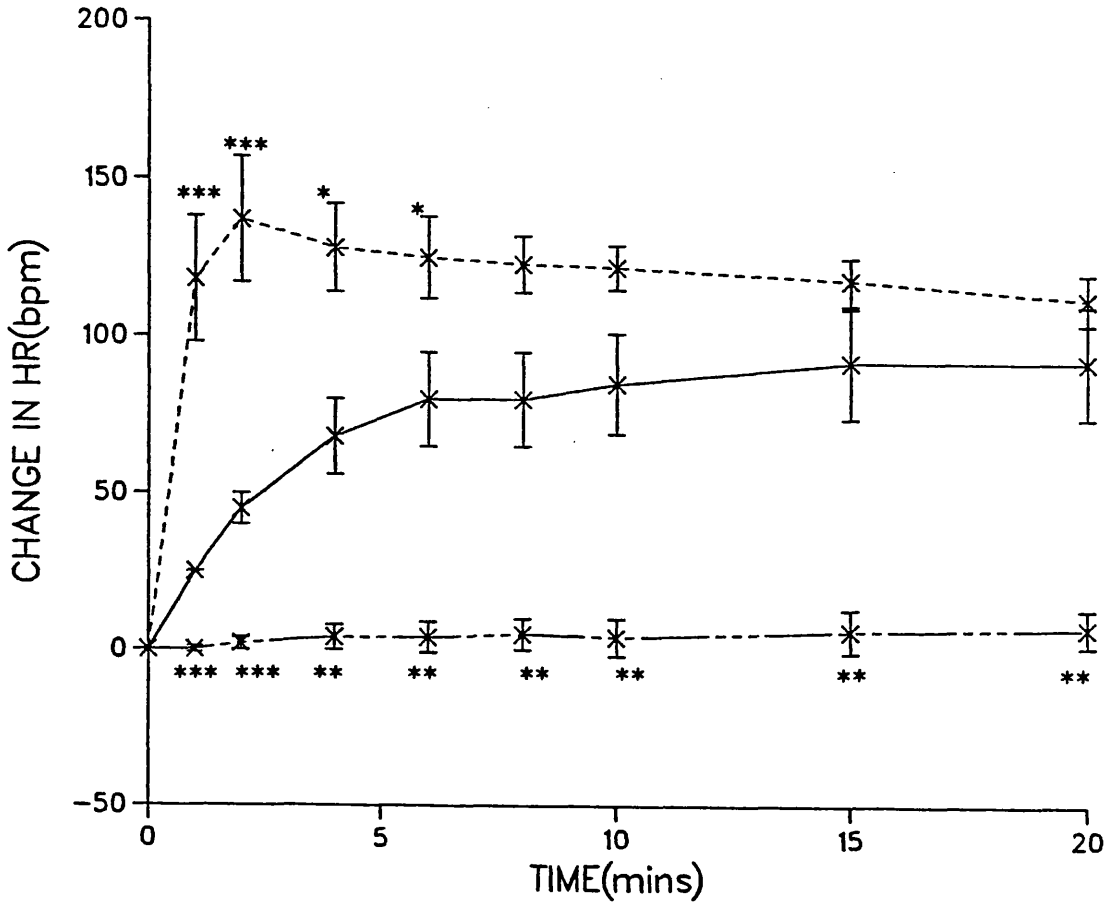

Figure 49b.

3.6.3. Discussion.

The fact that Inactin was used in this set of experiments precludes direct comparison of these results with those obtained in New Zealand rats since it is not known whether any discrepancy would be a result in strain difference or the use of a different anaesthetic. However, it was noted that pretreatment with icv propranolol did not significantly alter the hypotension produced by isoprenaline injected into the hypothalamus of New Zealand rats whereas the hypotension was significantly reduced in Japanese Okamoto rats and abolished in Wistar rats by pretreatment with icv propranolol. In all strains of rat studied, chronic oral dosing with propranolol attenuated both hypotension and tachycardia.

In Japanese Okamoto rats, oral dosage with propranolol abolished both hypotension and tachycardia induced by central injection of isoprenaline but icv injection of propranolol did not significantly attenuate the initial fall in blood pressure produced by isoprenaline. This would indicate that, in this strain of rat, it is possible that the injected propranolol had not been significantly concentrated in the hypothalamus as would be expected to happen following chronic oral dosing with propranolol. The results seen in Japanese Okamoto rats are comparable with those in New Zealand rats (see figures 48 & 49 and 33 &

34). In this study, no measurements of the concentration of propranolol in the hypothalamus were made, but this would be helpful in interpreting discrepancies in results between different routes of administration of propranolol pretreatment.

These results differed from those in Wistar rats where icv propranolol did block the isoprenaline induced hypotension. Chronic oral dosage with propranolol was more effective at blocking the responses to central isoprenaline in Japanese Okamoto rats than in Wistar rats. Whether this is a difference of any great importance has not been answered in this study, and clearly more work involving hypertensive and normotensive rats would have to be carried out before any definite conclusions could be drawn.

3.7. General Discussion.

One of the main problems in a study involving central administration of drugs is the difficulty in ascertaining the precise mechanism(s) involved in any response. There is discrepancy between results recorded by different authors depending upon species and/or strain used and whether anaesthesia is employed. In addition, studies involving icv injection necessitate investigation into whether the responses are centrally or peripherally mediated.

In this study, attempts were made to eliminate some of these problems. Although it was not possible to use more than one species of animal, three strains of rat were used to investigate any strain differences. Unfortunately, direct comparisons could not be made between all three since it was found that the anaesthetic employed initially was not suitable for the hypertensive strain. This problem was overcome to a certain extent by using the same anaesthetic for Japanese Okamoto spontaneously hypertensive and Wistar Kyoto normotensive rats. The problems associated with anaesthesia-induced responses were eliminated by using conscious New Zealand rats to study responses to icv injections. It would be useful to devise a method for injections into the hypothalamus of conscious rats since this would also serve to localise the area of

injection and minimise leakage to the periphery as was observed following icv injection in conscious rats. The method used for icv injection in conscious rats in this study would be unsuitable for injections into the hypothalamus because the wash-through volume would be too great for a localised injection.

As a major influence in this study was whether anaesthesia was present and what anaesthetic, if any, was used, it will be further discussed here.

Conscious vs. anaesthetised animals.

The term 'conscious' used to describe an animal's condition should be replaced by 'unanaesthetised' because the arousal state of the animal may vary. Changes in arousal state ranging from overt sedation to hyperexcitability may occur following the injection of substances into the central nervous system. It follows that, in conscious animals, changes in cardiovascular parameters may be secondary to a behavioural alteration. Very few studies in which conscious animals are used record any changes in arousal of the animal. Changes in behaviour in unanaesthetised dogs were recorded by Per Bolme et al (1967) following electrical stimulation of the hypothalamus. The first sign of behavioural reaction was the dog raising its head and looking around. Increasing intensity of stimulation led

progressively to anxiousness, excitement and rage. Clearly it can be seen that these changes will lead to a change in cardiovascular parameters which may not be a direct result of central stimulation. In this study, the animals were placed under the minimum amount of stress possible by leaving them unrestrained within the experimental cage. However, during the injection of drugs, the animals exhibited some degree of restlessness. Whether this was a cause of distress at being handled, as a result of the physical injection or a central effect of the drug is unknown. Following completion of the injection, the animals tended to settle down within the cage and remain relatively immobile over the remainder of the experiment. It follows that a slight increase in mean arterial pressure and heart rate would result from this initial period of restlessness, and this was indeed observed.

Regardless of these problems when working with conscious animals, those associated with anaesthesia are greater. The most widely recognised differential effect of anaesthetics is their greater suppression of cortical and diencephalic function than of medullary function; this could result in a systemic bias allowing the medulla an exaggerated role in the regulation of the cardiovascular system (Calaresu et al, 1975).

Gutman et al (1962) demonstrated an increase in blood

pressure following stimulation of the hypothalamus, mesencephalon and medulla of unanaesthetised rabbits. Following injection of pentobarbitone, these changes were suppressed or converted to hypotension. This suggested a preferential block of pressor centres by pentobarbitone.

The effects of different general anaesthetics on the electrophysiology of central neurones has been reviewed by Szabadi (1979). For example, chloralose markedly reduced the sensitivity of cortical neurones to acetylcholine and excitant amino acids whereas urethane, nitrous oxide and halothane had little effect. Excitatory responses to noradrenaline in the cortex are seen in animals anaesthetised with halothane, but are rarely seen when barbiturates are employed.

A more detailed discussion regarding the anaesthetics used in this study can be found in section 3.5.4.1.

The value of the anaesthetised preparation rests in the ability of the investigator to measure many variables under carefully controlled experimental conditions. Small changes in blood pressure and heart rate may be detected in the anaesthetised preparation since these parameters are very stable under such circumstances. These small changes can easily be masked in the conscious preparation by movement of the animal.

Leakage of drugs from the central nervous system to the periphery.

In an attempt to distinguish between centrally and peripherally mediated responses following icv injection, the amount of drug remaining in the brain was quantified using radiolabelled isotopes. Results from this study indicated that there was a large difference in the rate of disappearance of drug from the central nervous system between anaesthetised and unanaesthetised animals. Whereas 80% of the injected drug was still present in the brain after 20 minutes in the anaesthetised animals, only 20% still remained after 10 minutes in conscious animals. Although no studies have been made in anaesthetised animals, the results from conscious animals agree with the findings of Anderson et al (1977) in conscious rabbits and Smits and Struyker Boudier (1979) in conscious spontaneously hypertensive rats. The evidence indicates that not only is the presence of anaesthesia likely to alter the responses to icv injections, but also this difference in leakage from the brain would suggest a greater participation of peripherally mediated responses in conscious animals.

It is not known why there should be such a great difference between anaesthetised and unanaesthetised rats in the rate

at which drugs leak from the central nervous system to the periphery. There are possible reasons, such as changes in cerebrospinal fluid or blood flow, but these were not investigated in this study.

Although the physical natures of isoprenaline and propranolol are entirely different, e.g. lipophilicity, the two drugs were found to leak from the central nervous system to the periphery to approximately the same extent in both conscious and anaesthetised animals.

From these results, it would be easy to speculate that following icv injection in conscious rats, the responses observed would be peripherally mediated. This is not entirely true. The existence of a central component in the responses was demonstrated by the fact that, in conscious New Zealand rats, the hypotension produced by 5 mcg isoprenaline was abolished by 30 mcg propranolol icv, but not by 30 mcg propranolol iv (see figure 22a).

It remains to be demonstrated if the discrepancy between responses observed in conscious and anaesthetised animals is an effect of the anaesthetic or perhaps, at least in part, a result of this difference in leakage to the periphery.

Although authors have not investigated the amount of drug

leaking to the periphery in anaesthetised animals, many have abolished the responses to icv injection by spinal cord and vagus nerve transection indicating that insufficient drug had leaked to the periphery to exert an effect at peripheral adrenoceptors. This was demonstrated in anaesthetised dogs (Bhargava et al, 1972), cats (Gagnon and Melville, 1966) and rabbits (Toda et al, 1969). Thus the balance of evidence would indicate that this difference in rate of leakage of drugs to the periphery is present in other species in addition to the rat.

Possible further lines of research.

Many questions are still unanswered by this study and indicate further lines of research.

The experiments in this study were concerned only with the effects on mean arterial pressure and heart rate immediately following injection. There is evidence to suggest that, following central injection of drugs, changes in cardiovascular parameters may be seen after several hours. Following icv injection of propranolol, an initial increase in blood pressure followed by a longer lasting hypotension after several hours has been reported in conscious dogs (Conway and Lang, 1974), rabbits (Anderson et al, 1977; Dollery et al, 1973) and spontaneously hypertensive rats (Smits et al, 1979; Sweet and Wenger,

1976). It may be useful, therefore, to observe changes in cardiovascular parameters in conscious rats over a longer time period than was employed in this study.

The problems associated with the presence of anaesthesia and the leakage of drugs to the periphery in conscious animals indicate that injection of drugs into discrete brain areas of conscious rats would be useful since this would eliminate any alteration in response by anaesthetics and also minimise leakage of drug to the periphery. This would be particularly useful when considering injections of noradrenaline and adrenaline which interact with alpha-adrenoceptors. The anaesthetic used for New Zealand rats contains fluanisone which is known to block alpha-adrenoceptors (Peroutka et al, 1977). It is possible that the inclusion of fluanisone in the anaesthetic would abolish or attenuate responses which are a result of interaction at central alpha- adrenoceptors. The possibility of this occurring may also be investigated by using an alternative anaesthetic, such as thiobutobarbitone, although anaesthetics in general are thought to block pressor responses preferentially (Bergmann and Gutman, 1966; Gutman et al, 1962).

In order to facilitate injection into discrete brain areas in the conscious rat, a system would have to be developed to allow injection of very small volumes of vehicle

containing the required amount of drug. This would also allow injection of antagonist and agonist into the hypothalamus.

Injection of more than one substance into the hypothalamus would also be useful in anaesthetised rats. The system used in this study did not allow for this. It was found that, if the cannula was removed from the hypothalamus to reload with the agonist following injection of the antagonist, subsequent repositioning in the hypothalamus and injection of agonist resulted in leakage of the agonist along the track of the cannula. This did not appear to occur with a single injection and it was thought that on repositioning the cannula, the track through the brain was widened thus allowing leakage of injected vehicle. To try to overcome this, antagonist and agonist were injected together into the hypothalamus, but it was difficult to interpret the responses obtained and assign them to one or other of the components in the injection. It was decided to inject the antagonist into the cerebral ventricle whilst injecting agonists into the hypothalamus. However, it is not known to what extent the injected antagonist would be present in the hypothalamus at the start of injection of the agonist. It would be possible to evaluate the amount of propranolol in the hypothalamus following icv injection and oral dosing by administering the radiolabelled isotope and subsequent evaluation of radioactivity in the

hypothalamus.

Although a small amount of work was carried out with spontaneously hypertensive rats, this was by no means complete. In order to investigate any differences in the hypertensive animal, it would be useful to investigate responses seen in the conscious animal where the blood pressure and heart rate have not been depressed by the presence of anaesthesia. The possibility that alpha-adrenoceptors may play a part in the development of hypertension in the Japanese Okamoto spontaneously hypertensive rat (see section 3.5.4.1.) would suggest that central injection of substances that interact with alpha-adrenoceptors may illustrate a greater strain difference than injection of beta- adrenoceptor agonists.

Finally, it would be useful to investigate changes in blood pressure and heart rate independently of one another. The reason for this is it is difficult to assess whether an attenuation of hypotension is merely a result of potentiation of tachycardia as was seen in some experiments. Any increase in heart rate could be blocked by dosing animals with atenolol for 7 days as was employed when injecting isoprenaline into the anterior nucleus (see section 3.4.5.3. and figures 37a and 37b). Atenolol given as an oral dose is not thought to pass across the blood brain barrier to a significant extent due to its low

lipophilicity (Barrett, 1977) and would therefore not interfere with any centrally mediated responses occurring from subsequent central injections.

3.8. General conclusions.

From the results obtained in this study, it may be concluded that activation of central beta- adrenoceptors results in an increase in heart rate and a decrease in mean arterial pressure. The degree of hypotension can be attenuated or reversed by central beta- adrenoceptor blockade. Thus, it seems hard to understand how the central effects of beta- adrenoceptor blocking drugs could be beneficial in the treatment of hypertension. It is possible that in clinical use beta- adrenoceptor blocking drugs that are capable of crossing the blood brain barrier may exert an action within the central nervous system which is pro-hypertensive and actually opposes the other, antihypertensive actions.

However, it must be remembered that central alpha- and beta- adrenoceptors differ from corresponding peripheral adrenoceptors in a number of respects. Noradrenaline has been shown to produce both excitatory and depressant responses which appear to be mediated by pharmacologically distinct receptors; the excitatory responses by alpha-adrenoceptors and the depressant responses by beta-

adrenoceptors. There is also evidence that the functionally antagonistic alpha- and beta- adrenoceptors may occur on the same neurone. In some areas of the central nervous system neuronal responses to noradrenaline and isoprenaline can be blocked by both alpha- and beta-adrenoceptor blocking agents (Szabadi, 1979). Thus, care must be taken not to assume that central administration of beta- adrenoceptor agonists or antagonists will lead to solely an action on central beta- adrenoceptors.

From the results obtained here, it is clear that central beta- adrenoceptors do play a role in the maintenance of blood pressure, but many questions still remain unanswered.

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SUMMARY.

1. Icv injection of adrenaline in anaesthetised New Zealand rats resulted in an increase in blood pressure and bradycardia,whereas injection of drugs which exhibit agonist action only at β-adrenoceptors resulted in a fall in blood pressure with tachycardia. Adrenaline interacts with both α- and β-adrenoceptors, suggesting that central α-adrenocptors are concerned with hypertension and bradycardia whereas central β-adrenoceptors are concerned with hypotension and tachycardia. In addition, it would appear that under these conditions, α-adrenoceptor mediated responses predominate over β-adrenoceptor mediated responses. The hypotensive action of β-adrenoceptor agonists appears to be mediated via central β2-adrenoceptors, as the β2-adrenoceptor agonist clenbuterol causes hypotension and the β2-adrenoceptor antagonist ICI 118,551 blocked the hypotension caused by central isoprenaline. Inconsistent results in studies using the β1-adrenoceptor agonist xamoterol may have arisen because this drug is a partial agonist with some antagonistic properties. (See Table 1)

2. Injection of isoprenaline into the hypothalamus of anaesthetised New Zealand rats resulted in a fall in blood pressure with tachycardia. The hypotension was attenuated following pretreatment with icv atenolol and potentiated following ICI 118,551, suggesting that the hypotension was mediated via β1-adrenoceptors in the hypothalamus. However, hypotension was also elicited by clenbuterol injected into the hypothalamus. It is possible that clenbuterol and ICI 118,551 may be interacting with pre-synaptic β2-adrenoceptors, whereas atenolol may alter isoprenaline induced responses by blocking post-synaptic β1-adrenoceptors. Thus the possibility exists that both types of β-adrenoceptor are present in the hypothalamus and may be involved in the regulation of cardiovascular parameters. This could be confirmed by further investigations using selective β-adrenoceptor agonists and antagonists. (See Table 2)

3. Chronic oral dosage with propranolol blocked the responses to central isoprenaline more effectively in Japanese Okamoto rats than in Wistar rats. It is possible that, following long-term treatment with propranolol, adaptive changes take place which

would not be seen following acute pretreatment. In the hypertensive animal, these changes may occur more readily following long-term treatment with β-adrenoceptor blocking drugs, or may already be present as a consequence of hypertension. In addition, it should be noted that antihypertensive drugs are known to have a greater blood pressure-lowering effect in the hypertensive than in the normotensive state. (See Table 3)

Agonist	Adrenaline		Xamoterol		Isoprenaline				Clenbuterol			
	Anaesthetised		Anaesthetised		Anaesthetised		Conscious		Anaesthetised		Conscious	
Antagonist	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr
no pretreatment	↑	↓	↓↓	↑	↓↓	↑	↓↓	↑↑	↓↓	↑	↓↓	↑↑
propranolol icv	↑↑	↓	↑	↑	↓	↑↑	-	↑	-	↑	-	↑
propranolol iv			↑	↑↑	↓↓	↑↑	↓↓	↑↑↑	↓↓	↑↑		
propranolol po					↑	↑						
atenolol icv					↓↓	↑						
atenolol iv					↓↓	↑↑						
ICI 118,551 icv					-	↑↑						
ICI 118,551 iv					↓	↑↑						

Table 1.

Change in mean arterial pressure and heart rate following icv injection of adrenoceptor agonists in New Zealand rats: modification by pretreatment with β-adrenoceptor blocking agents.

↑ Slight change, ↑↑ Definite change, ↑↑↑ Pronounced change.

Agonist	Noradrenaline				Adrenaline				Isoprenaline				Clenbuterol			
	anterior hypothal		posterior hypothal		anterior hypothal		posterior hypothal		anterior hypothal		posterior hypothal		anterior hypothal		posterior hypothal	
Antagonist	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr
no pretreatment	↑↑	-	↓	↑↑	↓	↓↓	↓	↓↓	↓↓	↑	↓↓	↑	↓↓	↑	↓↓	↑
propranolol icv	↑↑	↓	↑↑	↑↑	-	↑	↑	-	↓	↑↑	↓↓	↑↑	↓	↑↑	↓	↑↑
propranolol po									↑↑	-	-	↑↑				
atenolol icv									↓	↑↑						
ICI 118,551 icv									↓↓↓	↑↑						

Table 2.

Change in mean arterial pressure and heart rate following injection of adrenoceptor agonists into the hypothalamus of New Zealand rats: modification by pretreatment with adrenoceptor blocking agents.

↑ Slight change, ↑↑ Definite change, ↑↑↑ Pronounced change.

Injection of Isoprenaline	icv				anterior hypothalamus				posterior hypothalamus			
	Wistar		JO		Wistar		JO		Wistar		JO	
Antagonist	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr	bp	hr
no pretreatment	↓↓	↑↑	↓↓	↑↑	↓↓	↑↑	↓↓	↑	↓↓	↑↑	↓↓	↑
propranolol icv	↓	↑	-	↑	↑	↑	↓	↑↑	-		↓	↑↑
propranolol po	-	↑	-	-	↓	↑	-	-	↓	↑	-	-

Table 3.

Change in mean arterial pressure and heart rate following injection of isoprenaline in Wistar and Japanese Okamoto (JO) rats: modification by propranolol.

↑ Slight change, ↑↑ Definite change, ↑↑↑ Pronounced change.

ABSTRACTS.

Draper A J, Redfern P H, Roberts J Cardiovascular changes following administration of adrenaline and isoprenaline to the hypothalamus of the anaesthatised rat. Br. J. Pharmacol 1987, 91, 385P

Draper A J, Redfern P H, Roberts J Hypotension induced by central injection of isoprenaline and clenbuterol: modification by central propranolol. Br. J. Pharmacol 1986, 88, 454P

CARDIOVASCULAR CHANGES FOLLOWING ADMINISTRATION OF ADRENALINE AND ISOPRENALINE TO THE HYPOTHALAMUS OF THE ANAESTHETISED RAT

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It has been shown that injection of adrenaline into the anterior hypothalamus caused an initial slight increase, followed by a longer lasting decrease, in blood pressure accompanied by a fall in heart rate (Struyker Boudier & Bekers, 1975). In these experiments we have compared the effects of adrenaline and isoprenaline injected into both the anterior and the posterior hypothalamus. Modification by prior icv propranolol was also investigated.

Male New Zealand normotensive rats (190–200g) were anaesthetised with Hypnorm/midazolam (Flecknell & Mitchell, 1984). The left carotid artery was cannulated to allow measurement of blood pressure. Guide cannulae were stereotaxically implanted according to the atlas on König & Klippel (1963). The agonists (5µg in 1µl artificial csf (Merlis, 1940) were injected into the hypothalamus over 2.5 min. Where appropriate, 30µg propranolol was injected icv in 10µl artificial csf 15 min before the agonist.

Table 1. Maximal change in mean arterial pressure (mmHg) and heart rate (bpm) following injection into the hypothalamus.
Mean ± sem (n) Difference from control * P<0.05, ** P<0.01 Student's t test

	Blood Pressure		Heart Rate	
	Control	Propranolol	Control	Propranolol
adrenaline, anterior nucleus	−9.8 ± 3.7 (12)	+4.2 ± 2.4 (6) *	−40 ± 8 (12)	+24 ± 6 (6) **
adrenaline, posterior nucleus	−19.8 ± 4.0 (8)	+25.2 ± 8.8 (6) **	−61 ± 16 (8)	−14 ± 7 (6) **
isoprenaline, anterior nucleus	−15.5 ± 3.4 (11)	−12.0 ± 6.5 (6)	+23 ± 8 (11)	+61 ± 3 (6) **
isoprenaline, posterior nucleus	−19.5 ± 4.8 (9)	−24.0 ± 8.0 (6)	+14 ± 3	+51 ± 7 (6) **

Pretreatment with propranolol abolished the depressor response to adrenaline in the anterior nucleus, and reversed the response in the posterior nucleus. The depressor response to isoprenaline was unaffected by pretreatment with propranolol, although the duration of hypotension was attenuated.

Previous studies involving icv injection of isoprenaline have indicated that the central depressor response consists of two elements, one mediated through β-receptors and the other involving non-neuronal mechanisms (Peres-Polon & Correa, 1984; Draper et al, 1986). These results appear to support this hypothesis, since the depressor response to adrenaline is readily abolished or reversed by propranolol whereas the magnitude of the depressor response to isoprenaline in unaffected.

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Peres-Polon VL, Correa FMA, (1984) Gen. Pharmacol. 15, 505–509
Draper AJ, Redfern PH, Roberts J, (1986) Br. J. Pharmac. 88, 454P

HYPOTENSION INDUCED BY CENTRAL INJECTION OF ISOPRENALINE AND CLENBUTEROL: MODIFICATION BY CENTRAL PROPRANOLOL.

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Hypotension has been reported to follow icv injection of isoprenaline in anaesthetised animals of various species (Gagnon & Melville, 1967; Toda et al, 1969; Bhargava et al, 1972). In these experiments we have attempted to elucidate the mechanism involved by comparing the effects of two β-adrenoceptors agonists, isoprenaline and clenbuterol, administered centrally to the anaesthetised rat. Modifications of their effects by prior central and peripheral injection of the β-adrenoreceptor antagonist, propranolol, was also investigated.

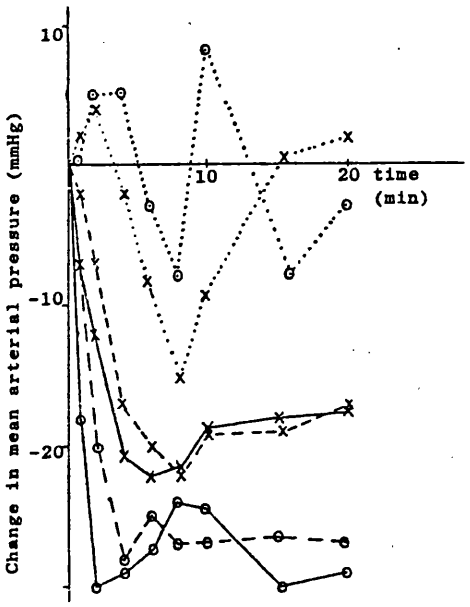

Fig. 1. Change in mean arterial pressure produced by ICV isoprenaline 5μg (x——x) and clenbuterol 5μg (o——o). Modification by propranolol 30μg ICV (x....x, o....o) and 12μg iv (x----x, o----o). (n = 6).

Male New Zealand normotensive rats (190-200g) were anaesthetised with Hypnorm/midazolam (Flecknell & Mitchell, 1984). The left carotid artery and right jugular vein were cannulated to allow measurement of blood pressure and injection of drugs respectively. A guide cannula was inserted into the left cerebral ventricle and drugs were injected icv in a volume of 5μl over 2.5min.

The dose-dependent hypotension produced by both isoprenaline and clenbuterol icv was resistant to prior injection of 12μg propranolol iv (Fig.1). Pretreatment with 30 g propranolol icv 15 min before the agonist abolished the depressor response to clenbuterol, but only partially prevented the hypotension produced by isoprenaline (Fig. 1). This confirms earlier reports (Nomura, 1976; Peres-Polon and Correa, 1984) that in the anaesthetised rat, the depressor response to central isoprenaline is resistant to propranolol, suggesting that a significant element of the depressor response of isoprenaline is not mediated through β-adrenoceptors. There is, of course, always the possibility, following icv injection, of leakage of drug to the periphery. However, central injection of ^{3}H-propranolol and ^{3}H-isoprenaline with subsequent analysis of blood and tissue isotope levels indicated that, for instance, in terms of the amount of drug available to the periphery, 12μg propranolol iv was equivalent to 30 μg propranolol icv.

Taken together, these results support the hypothesis that the central depressor response to isoprenaline comprises two elements; one mediated through β-adrenoceptors and mimicked by clenbuterol and the other probably mediated through humoral mechanisms.

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